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CHEMICAL COMPOSITION OF ROOTS AND DECOMPOSING ROOT RESIDUES FROM THREE GRASS SPECIES

by

© WILLIAM ALLAN HERMAN

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF SOIL SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Chemical Composition of Roots and Decomposing Root Residues from Three Grass Species", submitted by William Allan Herman, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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Date Dec. 21, 1973.

ABSTRACT

Experiments were conducted to determine the influence the chemistry of the roots of three prairie grasses might express in the rate of root decomposition and in the types of products formed.

Roots of Festuca scabrella (rough fescue), Stipa spartea variety curtiseta (western porcupine grass) and Stipa comata (spear grass) were collected from the Thin Black, Dark Brown and Brown soil zones, respectively, of Southern Alberta. These were incubated at 28°C in liquid culture and aerated by bubbling CO_2-free air through the liquid. The content of carbon, nitrogen, hydrogen and phosphorus in the undecomposed root tissue was measured, as was the content of soluble and structural carbohydrates, amino acids and lignin. Numbers of carboxyl and methoxyl groups were also determined. After various periods of decomposition, the water insoluble residues were similarly characterized. The water soluble fractions were examined spectroscopically but due to limited amounts of sample were not as completely characterized chemically.

The greatest amount of decomposition, in terms of loss of original root weight, occurred in the roots of S. spartea which lost 40 percent of its ash free weight followed by F. scabrella and S. comata losing 34 percent and 31 percent, respectively. The loss of carbon by each species did not

follow this trend. The C/N ratio of the raw roots of each species was related to the loss of carbon but not the loss in root weight. The raw roots of F. scabrella, S. spartea and S. comata were found to have C/N ratios of 35/1, 22/1 and 18/1, respectively, and experienced a carbon loss of 32 percent, 28 percent and 33 percent, respectively.

The highest amino acid content in the raw roots was associated with the greatest carbohydrate loss and least lignin loss.

Examination of spectroscopic data (infrared, ultraviolet and visible) would indicate no substantial changes in the water insoluble materials with degradation mostly occurring in aliphatic groups. Small increases in the C/H ratios of the root residues support this data. Increasing E4/E6 ratios of water soluble materials was considered an indication of decreasing chemical complexity in the water soluble fraction and possible conversion of aromatic material into water insoluble material.

The incubation period, 47 weeks, and/or the conditions for decomposition were insufficient for complete humification of the roots and residues.

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INTRODUCTION

The four soil zones of Southern Alberta are differentiated by soil color, organic matter content and in the case of the Black and Thin Black Soil Zones, the depth of the Ah horizon. Progressing from east to west across the province, soil color generally darkens and organic matter content of the surface horizon increases as one passes through the Brown, Dark Brown and Thin Black Soil Zones.

Differences in the chemical character of the decomposed plant material at sites in each soil zone might be attributed to differences in climate, annual plant material additions, the influence of the soil mineral fraction and/or differences in the initial chemistry of the plant species growing at each site.

The roots of three common prairie grasses, Festuca scabrella (rough fescue), Stipa spartea variety curtiseta (western porcupine grass) and Stipa comata (spear grass), were sampled from the Thin Black, Dark Brown and Brown Soil Zones, respectively. The undecomposed roots were chemically characterized and allowed to decompose under laboratory conditions. At various intervals, the decomposing roots were again chemically characterized to attempt to determine the influence of the chemical composition and associated microorganisms of the initial root materials on:

(a) the rate of decomposition of each grass species; and (b) the products of decomposition of each grass species.

LITERATURE REVIEW

Products formed during decomposition of plant material in soil are partitioned into three main categories. A portion is used for synthesis of biomass, while a portion is completely mineralized to CO_2, NH_4^+, $SO_4^=$ and $PO_4^{\equiv}$. The third category is the humic material formed through reactions between extra cellular microbial metabolites, partially degraded plant components and existing organic and mineral entities in soil.

Composition of Plant Tissues

Plants generally contain the same classes of compounds (waxes, fats, resins, proteins, simple and complex carbohydrates, lignin and other components) but the proportions depend on the species being considered (Table 1). The proportions of the various constituents present influences the degree and rate of decomposition (Table 2). This fact helps account for different rates of decomposition of different plant species (Table 2).

Cellulose is a major constituent (25 to 30 percent) of perennial grasses (Table 1). It is present in plants as coiled ribbon-like structures composed of a minimum of one thousand D-glucose molecules linked through β1-4 oxygen

Table 1. Approximate Chemical Composition of Higher and Lower Plant Organisms*
(as percent of dry matter)

Organisms Investigated	Waxes Fats Resins	Protein	Cellulose	Hemicelluloses and Soluble Carbohydrates	Lignin
Perennial leguminous plants					
Roots	10-12	10-15	20-25	25-30	10-15
Leaves	-	12-20	15	10-12	5
Perennial grasses					
Roots	5-12	5-10	25-30	25-30	15-20
Deciduous species					
Leaves	3- 5	4-10	15-25	10-20	10
Wood	-	0.5- 1	40-50	20-30	20-25
Coniferous species					
Needles	20-25	5- 7	20	15-20	15
Wood	-	0.1- 1	45-50	15-25	25-30
Mosses	-	5-10	15-25	30-60	None (?)
Lichens	-	3- 5	5-10	60-80	8-10
Algae	-	10-15	5-10	50-60	None
Bacteria	-	40-70	None	Slime	None

* Kononova, M. M. 1966. Soil organic matter. p. 112.

Table 2. Appearance of Humus Production and Chemical Composition of Plant Residues*

	Clover Leaves	Clover and Lucerne Roots	Agropyron tenerum Roots	Agropyron cristatum Roots	Hazel Leaves	Scots-pine Needles
The beginning of visible changes (days)	2- 4	5- 8	20- 30	20- 25	5- 8	30- 35
The first appearance of humus substances (days)	14-20	60-75	180-200	180-200	25-30	120-180
Constituent						
Fats, Waxes and Resins	23.07	11.24	6.97	No Data	No Data	24.47
Starch	3.00	17.75	None			None
Hemicellulose	8.07	11.94	22.86			12.68
Cellulose	15.40	20.97	25.49			27.59
Protein (N x 6.25)	21.67	13.31	7.94			6.97
Lignin	4.29	8.61	18.43			15.05
Total	75.5	83.82	81.69			86.76

* Kononova, M. M. 1966. Soil organic matter. p. 158-159.

linkages. It is often closely associated with other plant polysaccharides by absorption or secondary valency forces (Gupta, 1967). This association and the coiled nature of the cellulose molecule imparts resistance to chemical and microbial attack.

In combination with cellulose, lignin gives strength and durability to plant cell walls. Perennial grass roots contain 25 to 30 percent lignin (Table 1). Only coniferous and deciduous wood has more lignin. The precise chemical structure of lignin is not known, but it is considered to be a complex macromolecule with variable chemical characteristics and types of bonds (Oglesby, Christman and Driver, 1967). Flaig (1964) describes lignin of perennial grasses as basically p-coumaryl alcohol units linked through aliphatic ether groups, aryl alkyl ether groups, diphenyl linkages and carbon of side chains linked with aromatic ring carbons. He describes coniferous tree lignin as having a coniferyl alcohol building unit while deciduous tree lignin being composed of coniferyl alcohol and sinapyl alcohol units (Flaig, 1964). Barghoorn (1952) reported that plant lignin content was correlated with slow decomposition rates; the complex lignin macromolecule being most slowly attacked by microbial enzymes. The large number and type of bonds randomly distributed in the lignin molecule renders it the most resistant plant constituent. With no nitrogen in the

structure, other sources of nitrogen must be available for microbial degradation of lignin.

Hemicellulose, another constituent of uncertain structure, is found in plant cell walls in association with lignin as structural strength yielding units (Gupta, 1967). Our incomplete understanding of the structure of hemicellulose is due in part to difficulties in removing the hemicellulose envelope from the cellulose coils it coats (Gupta, 1967). Hydrolysis of hemicellulose yields D-glucose, D-glucuronic acid, D-xylose, D-galactose, D-galacturonic acid, L-arabinose and D-mannose.

Protein constitutes 5 to 10 percent of the weight of perennial grass roots (Kononova, 1966, p. 112). In plants, proteins serve both as enzymes and as structural units. In primary cell walls, the protein portion serves to coat the lipid bilayer facilitating proper orientation of the two lipid layers. The lipid-protein association would be expected to render cell wall protein more resistant to degradation than is enzymic or cytoplasmic protein. To date, little information concerning the relative distribution of enzyme and structural protein is available.

Plant waxes (esters of higher fatty acids and aliphatic alcohols), fats and resins serve in plant tissues as coatings to insure a satisfactory moisture balance in leaves and stems. Kononova (1966, p. 112) reports a 5 to 12 percent fats, waxes and resins content in perennial grass

roots. Although their role in root tissues is not well understood, they may protect against disease invasion (Stumpf, 1965).

Microorganisms Involved in Decomposition

The varied physical and chemical characteristics of soil and the diverse nature of plant materials added to it, results in a large and varied population of soil microorganisms which decompose plant tissues (Alexander, 1961, p. 3).

Alexander (1961, p. 168-169) considers fungi to be the main cellulose degraders in humid soils while bacteria are of greater significance in semi-arid soils. The large number of microorganisms capable of attacking cellulose permits its decomposition through a wide range of pH levels, moisture conditions, temperatures and oxygen tensions (Table 3). Alexander (1961, p. 167) states that degradation of cellulose in soil is a joint effort by a broad spectrum of microorganisms, with more rapid degradation in a mixed culture than a pure culture.

Two major problems have been encountered in attempts to identify lignin degrading microorganisms. These are slow rates of chemical change during decomposition and the difficulty in extracting lignin without chemical alteration. Soil fungi, particularly the Basidiomycetes and Ascomycetes,

Table 3. Some Microbial Genera Capable of Utilizing Cellulose*

Fungi		Bacteria		Actinomycetes
Alternaria	Polyporus	Achromabacter	Clostridium	Micromonospora
Aspergillus	Rhizoctonia	Angiococcus	Cytophaga	Nocardia
Chaetomium	Rhizopus	Bacillus	Polyangium	Streptomyces
Coprinus	Trametes	Cellfalcicula	Pseudomanas	Streptosporangium
Fomes	Trichoderma	Cellulomonas	Sorangium	
Fusarium	Trihothecium	Cellvibrio	Sporocytophaga	
Myrothecium	Verticillium		Vibrio	
Penicillium	Zygorhynchus			

* Alexander, M. 1961. Soil microbiology. p. 169.

are the main degraders of lignin; their mycelia are capable of penetrating the ligno-cellulose association (Garret, 1934). Alexander (1961, p. 206) reports a study in which 44 of 46 soil Basidiomycetes studied were capable of degrading both cellulose and lignin.

Many soil microorganisms utilize hemicellulose as a carbon and energy source (Table 4). Due to the heterogeneity of hemicellulose hexosan and pentosan components, the rate of degradation is not constant. Experiments have shown hemicellulose to stimulate growth of cellulose degrading microorganisms (Alexander, 1961, p. 188).

Proteins are readily attacked by many soil microorganisms. Peptide linkages between amino acids are hydrolysed by proteolytic enzymes, the proteases. The products of degradation of proteins, peptides and amino acids may persist in soil through complexing with clays and aromatic products of lignin degradation. Free amino acids are rare as they are rapidly degraded by soil microorganisms.

Factors Influencing Decomposition Rate

Study of the decomposition rate of individual plant components has been limited by the difficulty in extracting the components in a pure state (Jenkinson, 1971). The period of residence, in an unchanged state, of each plant component

Table 4. Microorganisms that Utilize Hemicelluloses*

Organism	Substrate
Bacteria	
Anaerobes	Galactan, mannan
Bacillus	Xylan
Bacillus, *Achromabacter*	Oat hemicellulose
Bacillus, *Pseudomonas*	Wheat pentosan
Cytophaga, *Sporocytophaga*	Hemicellulose
Lactobacillus	Xylan
Vibrio	Mannan, xylan
Actinomycetes	
Actinomycetes	Galactan, mannan, xylan
Streptomyces	Wheat pentosan
Streptomyces	Oak hemicellulose
Fungi	
Alternaria, *Fusarium*, *Trichothecium*	Hemp polyuronide
Aspergillus, *Rhizopus*, *Zygorhynchus*	Galactan, mannan, xylan
Chaetomium, *Helminthosporium*	
penicillium	Wheat pentosan
Coriolus, *Fomes*, *Polyporus*	Araban, galactan, mannan

* Alexander, M. 1961. Soil microbiology. p. 189.

must be viewed in terms of the multiplicity of potential chemical reactions in the soil environment.

The association of two or more cellular components reduces the rate of decomposition of each component (Table 5).

Table 5. Decomposition by Pseudomanas ephemerocyanea of Jute Preparations Containing Different Quantities of Cellulose and Lignin*

Preparation	Cellulose Content %	Lignin Content %	% of Cellulose Decomposed
A	99.2	0.0	100.0
B	95.5	3.3	95.6
C	89.2	6.3	83.1
D	82.7	11.9	37.9
E	75.6	12.9	17.7

Incubation period of 21 days.

* Alexander, M. 1961. Soil microbiology. p. 167.

The reduction in decomposition rate may be attributed to an increased number of bonds to break between components and an increased complexity of bonds requiring complex enzyme systems (Estermann, 1959). Component associations, such as cellulose-

lignin complexes, may sterically inhibit the activity of lignin or protein degrading enzymes through prevention of proper enzyme-substrate contact (Swaby, 1968). Swaby (1968) also suggests organic crypts, a component existing within another organic entity, may prevent enzymic attack.

In attacking any substrate for energy and eventual new cell production, sufficient nutrients must be available to the microorganisms before any major alterations may occur. Nitrogen levels are of particular importance in the degradation of carbohydrates for eventual protein synthesis.

The production of toxic metabolic products of decomposition may slow a constituent's degradation (Alexander, 1965).

Clays may strongly influence the degradation of plant constituents by adsorbing the constituent and/or the enzyme. The reduced mobility of the substrate-enzyme system may prevent proper contact for reaction.

Swaby (1968) notes that orderly polymers with regularly repeating monomer units, such as cellulose, depolymerize more rapidly than those molecules lacking order, such as lignin. The variable nature of bonding necessitates more than one enzyme for degradation.

The environment must provide proper conditions of oxygen tension, temperature, pH and moisture conditions for healthy microbial activity. Any one or more of these conditions

lacking in the environment may eliminate a required organism and its enzyme system for efficient decomposition of plant tissues (Swaby, 1968).

Decomposition Products and Fate

A discussion of plant tissue decomposition must stress the possibility that any constituent may participate in soil humus formation; any plant constituent may be totally mineralized and not participate. The environmental conditions for decomposition select the role of each constituent (Aleksandrova, 1972).

Analyses of soil organic matter has often shown a cellulose content of less than one percent (Gupta, 1967), despite a high cellulose content in living plant tissues (Table 1). These results imply cellulose being completely mineralized and used as an energy source, being used in microbial cell walls in the rod-shaped micelle form or used in the synthesis of new organics appearing in soil organic matter (Alexander, 1961, p. 163). Cellulose is hydrolysed by cellulase enzymes to yield the disaccharide cellobiose. Cellobiose is hydrolysed by the enzyme cellobiose to yield two glucose molecules. Alexander (1961, p. 173) lists carbon dioxide, hydrogen, ethanol, acetic acid, formic acid, succinic acid, butyric acid and lactic acid as products of complete anaerobic

cellulose decomposition. He lists carbon dioxide and water as aerobic products.

Alexander (1961, p. 200) suggests the outstanding microbial characteristic of lignin is its resistance to degradation; lignin being the last plant constituent to show appreciable oxidation. Flaig (1964) suggested the probable absence of nitrogen in the lignin molecule, thereby an unfavorably high C/N ratio, limits the activity of lignolytic microorganisms. This situation is also the case with cellulose and hemicellulose and their respective microbial degraders. Flaig (1964) added ammonium nitrate-nitrogen to accelerate the decomposition of lignin. The source and amount of nitrogen available to soil microorganisms is of great importance. Research may show a high amino acid level in microorganisms to be necessary for rapid decomposition of plant tissues.

Alexander (1961, p. 200) views the initial decomposition of lignin as a modification of the macromolecule including cleavage of three carbon sidechains from their aromatic nucleus and a decrease in methoxyl group content. The number of aromatic hydroxyl and carboxyl groups increases. The large size of the lignin macromolecule necessitates extra cellular enzyme catalysis. After most plant celluloses, hemicelluloses and proteins have decomposed, slow changes in lignin occur. The changes may include degradation of the macromolecule to its constituent phenylpropane building

units, oxidation of the benzene ring to quinoid structure and polymerization of the quinones to a highly resistant entity.

Hemicellulose, also requiring extracellular enzymic attack, degrades to its constituent hexosan and pentosan sugars used as energy sources and cell synthesis (Gray and Williams, 1971).

Amino acids, the decomposition products of peptides and proteins may display unexpected long residence periods in soil due to complexing with soil clays. Sorensen (1971) attributes an unexpected long period of residence of amino acids in soil to adsorption of peptides and proteins to clay particles. Subsequent enzyme deactivation may occur through reduced mobility of substrate and enzyme (Marshall, 1964). Soil amino acids eventually degrade with ammonium being released as a product (Alexander, 1961, p. 253). Bremner (1955) reported 20 to 50 percent of organic bound soil nitrogen to be amino acid nitrogen.

Concepts of Humification

The degradation of plant tissue constituents proceeds with a fraction of the products being used for microbial cell synthesis, a fraction being totally degraded to a mineralized state while the remainder is found in

resistant soil organic matter termed humus. Humus materials exist in soil for long periods of time due to resistance to enzyme attack. The random nature of bonding in humus and the potential for adsorption to clay particles results in impeded enzyme attack.

Through complex physio-chemical changes, all plant constituents may contribute to soil humus (Kononova, 1966, p. 181). Kononova (1966, p. 117) reviewed research by Mulder and Liebig who produced humus-like substances through the treatment of wood, containing 25 percent lignin, with alkali solutions. Decomposition of cellulose and hemicellulose alone failed to yield brown or black colored humus products. Hoppe and Seyler found addition of whole wood to the cellulose plus hemicellulose medium resulted in the microbial synthesis of humus material.

The complex reactions in soil leading eventually to soil humus should not be associated exclusively with the lignin fraction. Although generally much less resistant to microbial attack, all other plant constituents may play a role in the formation of soil humus. Sterically protected by organic macromolecules preventing microbial or enzymic contact or adsorbed by colloids may serve individually or collectively to increase the residence time of an organic material in soil (Swaby, 1968).

Oglesby, Christman and Driver (1967) proposed a simplified pathway for humification which considers lignin

to be the major contributor to the humus product. Through microbial attack, the lignin macromolecule is degraded to its phenyl propanoid units. These are enzymically oxidized to highly reactive quinoid structures capable of polymerization to form the condensed humus macromolecule. The polymerization of aromatic quinoid structures includes condensation with peptides, a product of plant protein degradation. The product exhibits highly random bonding of aromatic rings. The randomness of the bonding may inhibit specific enzyme substrate site conformation resulting in slow decomposition (Mahler and Cordes, 1968).

Kononova (1966, p. 150) suggests a second scheme representing soil humus production (Figure 2). This scheme differs from that of Oglesby, Christman and Driver's (Figure 1) in that it includes plant proteins, carbohydrates and the microbial metabolic products in the synthesis of humus in addition to the products of lignin degradation.

Microbial phenoloxidase enzymes are important in the oxidation of phenolic structures to quinoid structures (Kononova, 1966, p. 142). The reaction diagrammed in Figure 3 procedes easily at alkaline pH in the presence of phenoloxidases derived from fungi and actinomycetes. The eventual products are dark colored polymers (Kononova, 1966).

A more recent attempt at understanding soil humus formation has developed through electron spin resonance (E.S.R.) counting of free radicals. A free radical is a

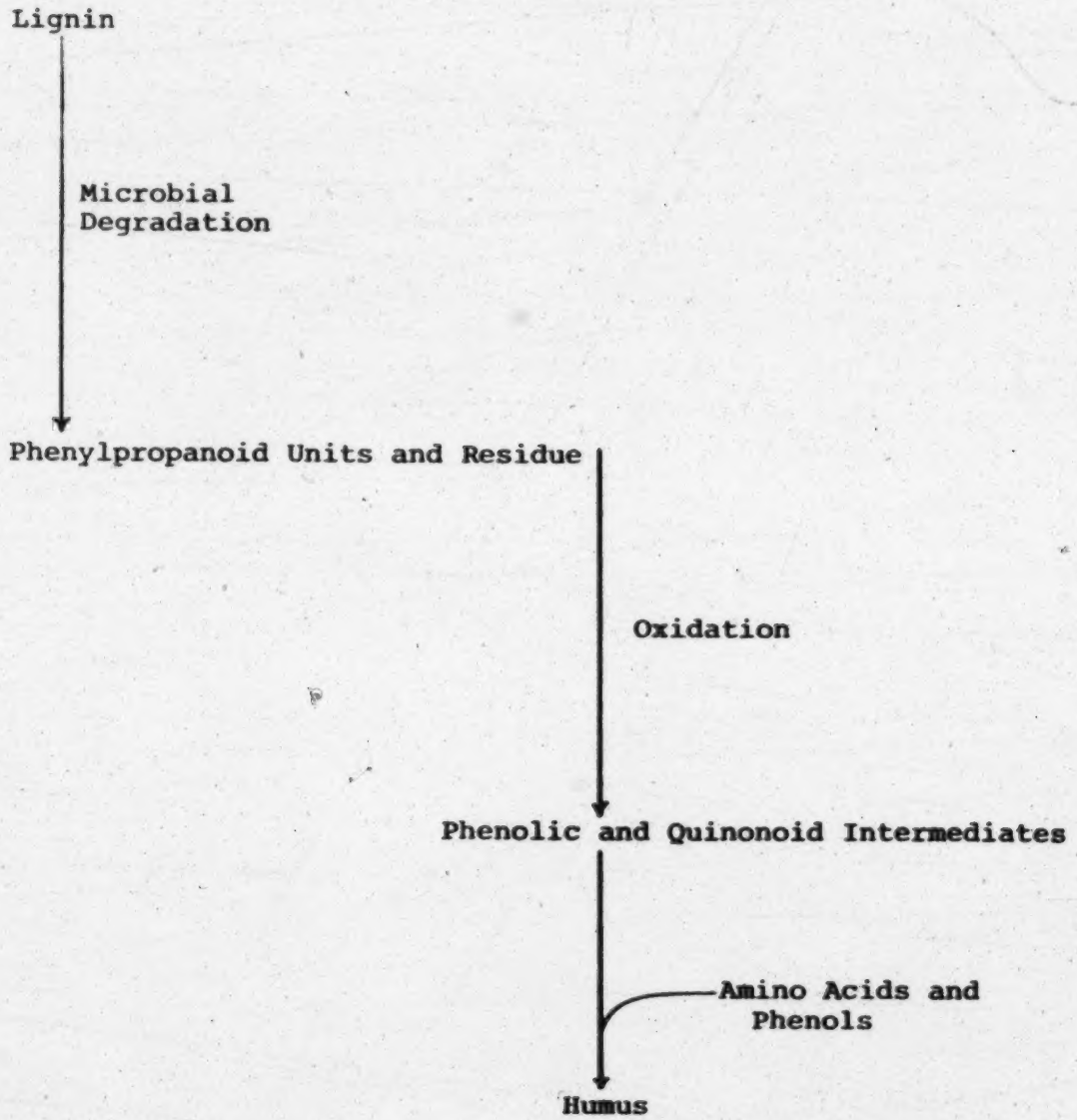


Figure 1. Scheme for humus production from lignin as proposed by Oglesby, Christman and Driver.

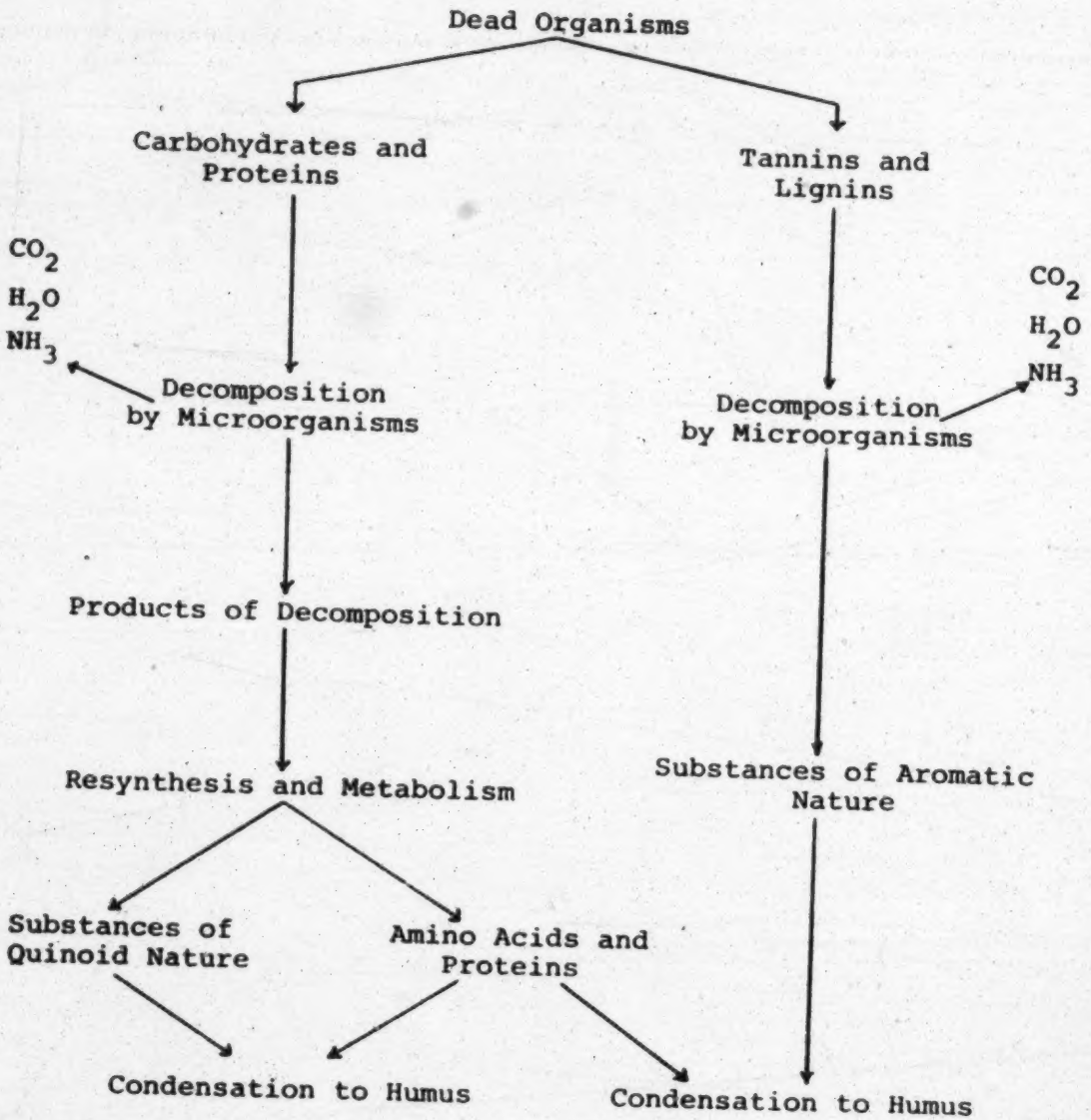


Figure 2. Scheme for humus production as proposed by Kononova.

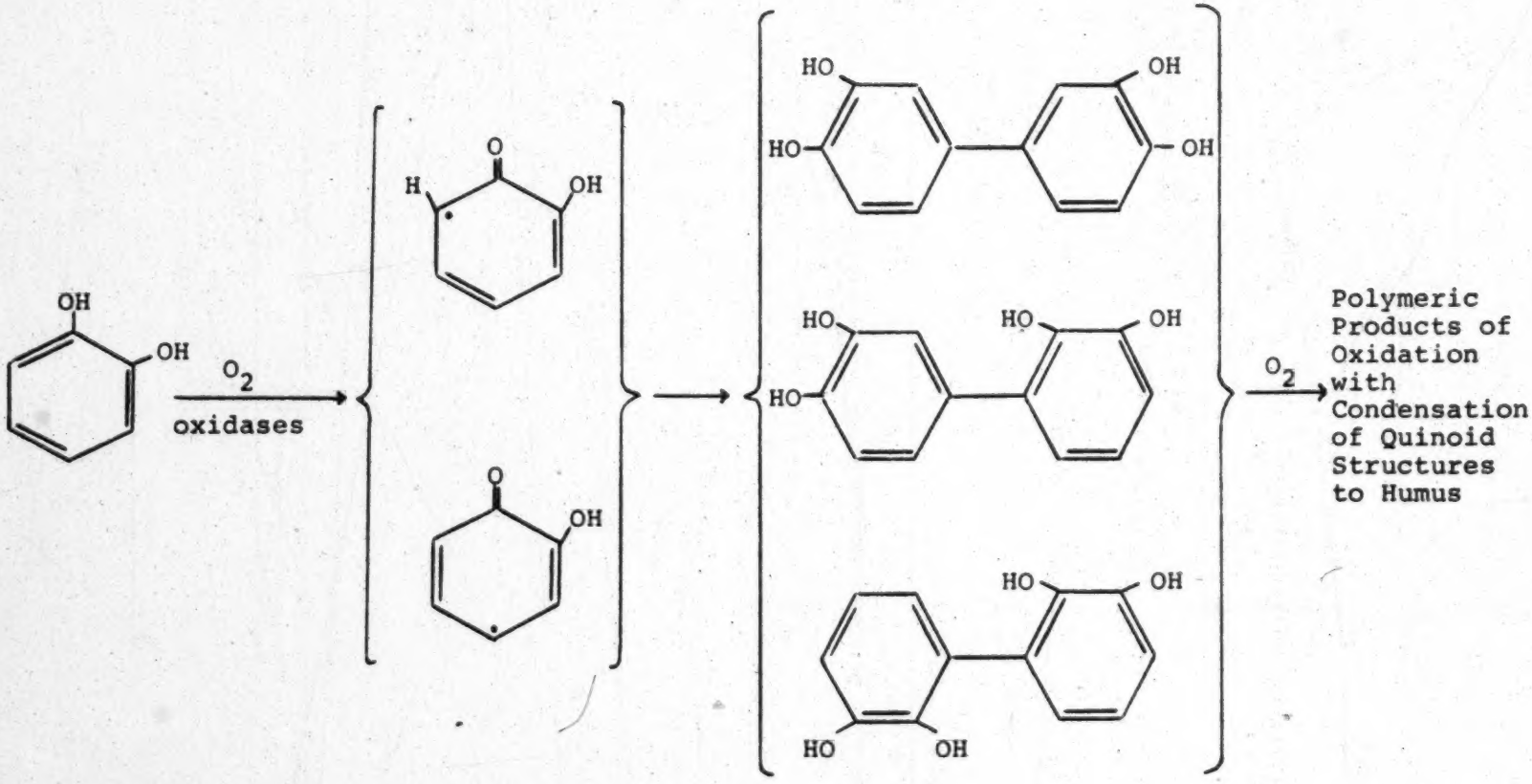


Figure 3. Scheme for production of polymers from quinoid structures proposed by Kononova.

stable molecule with an unshared electron; the spin of that electron being countable.

Free radicals may be stabilized within plant lignin by being trapped or caged with a complex organic network (Harkin, 1967). Atherton (1966) rejects the caged radical concept feeling humus itself is a large free radical gaining unusual stability through an extended conjugated system. Lone electrons may be stabilized through extensive resonance in pi orbitals of aromatic ring structures of soil organic matter. Atherton's concept may over emphasize electron sharing by resonance. For sharing throughout the entire organic molecule, all ring structures must be flat and in the same plane. Viewing the entire humus molecule as a free radical cannot totally explain the stability of the unshared electron. Atherton (1966) suggests the lone electron, giving electron spin properties, originates from an oxygen atom. The electron spends less time in the pi orbital of the oxygen parent atom as addition and aromatic rings are added or condensed to the complex.

According to Schnitzer (1972), the structure and origin of soil organic matter free radicals is not fully understood. Steelink and Tollin (1961) have shown the free radical concentration to increase with passing decomposition time; their research favoring a quinone group accepting a single electron from amines or phenolic hydroxyls to form a semiquinone, a stable free radical. Caution should be

exercised as there is no absolute proof of substantial quantities of quinone groups existing in soil organic matter. Research by Rex (1960) showed free radicals to be stable in soil for many years despite exposure to oxygen. Rex (1960) failed to find free radicals in living plant tissues. Rex (1960) suggests that free radicals in soil organic matter are a result of acid or fungal attack on plant protein-lignin complexes.

Haworth (1971) states useful structural deductions cannot be made from current electron spin resonance data. He feels much work remains to be done in evaluating E.S.R. as a tool for the understanding of the structure of soil humus.

After the death of a cell, intracellular enzymes are still active before microbial attack. Swaby and Ladd (1965) have hypothesized humic substances may be the product of heterogeneous chemical catalysis; the condensation or polymerization of free radicals formed enzymatically in plant and microbial cells after death.

In investigating the various concepts and techniques of analyzing soil organic matter to gain an understanding of the synthesis of soil organic matter, the researcher must constantly be aware of the complexity of the soil system. The soil environment is capable of many chemical reactions, many organic systems and products may exist in the soil.

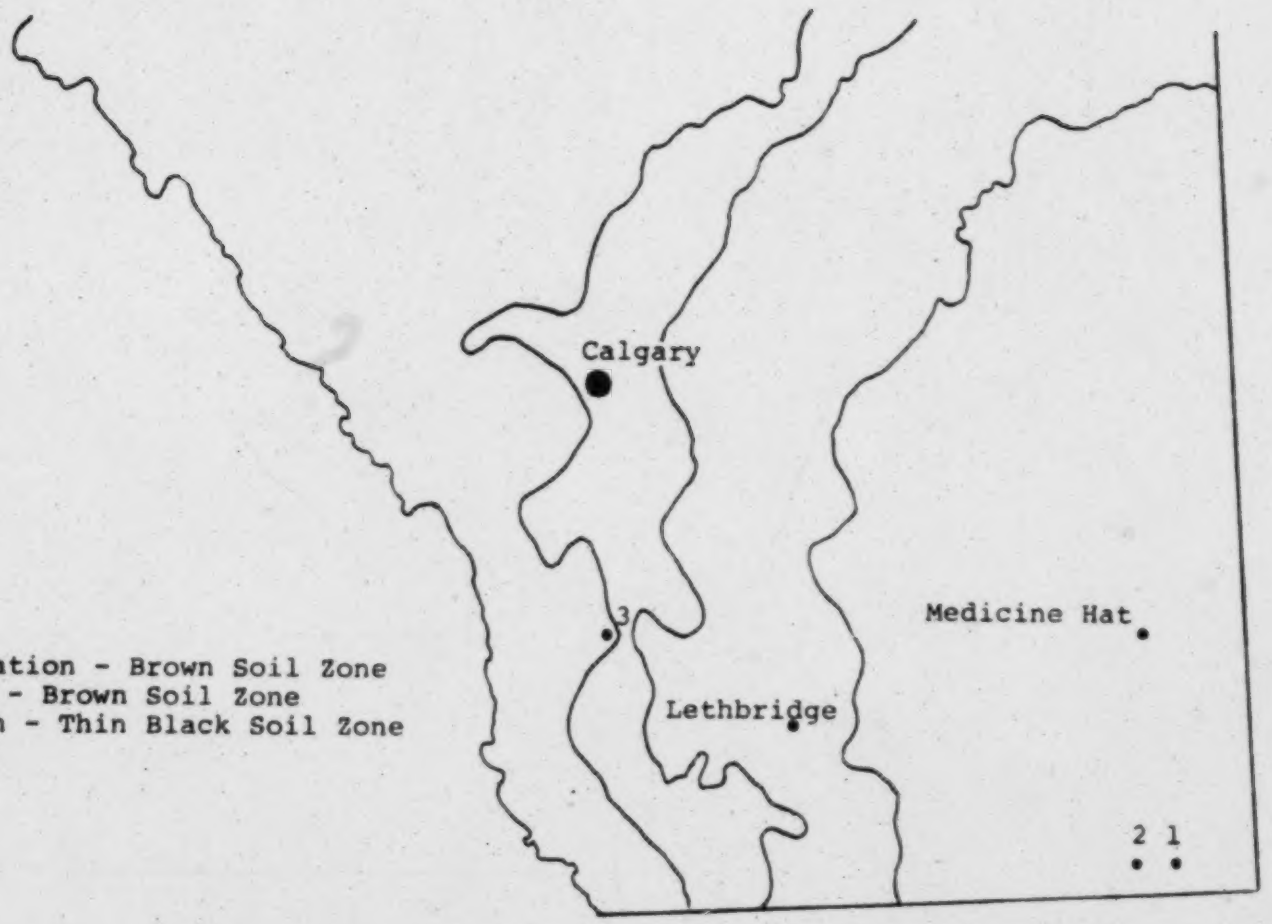
Many researchers have proposed new systems for the synthesis of soil humus. No single scheme has been totally accepted; an element of probability exists in all proposed schemes. An element of frustration exists in studying soil humus and plant lignin extracts without chemical alteration. To date, all lignin or humus extracts contain artifacts.

It is often difficult to gather information specifically dealing with root tissue degradation. Relative to studies of root decomposition, past research has emphasized the importance of plant surface growth.

MATERIALS AND METHODS

Site and Plant Community Description

Soil samples containing desired plant roots were gathered in May, 1971, from three sites in Southern Alberta (Figure 4). The three sites were selected to include three dominant plant species and three soil Great Groups (Table 6). The plant species selected were Stipa comata Trin. & Rupr., Stipa spartea Trin. variety curtiseta Hitchc. and Festuca scabrella Torr. and were present in soil of the Brown, Dark Brown and Black Great Groups, respectively (Figure 4, Table 7 and Plates 1 to 9). Samples of the Brown soil Great Group were taken at the Onefour Research Substation from SE 16-2-4 W5. This is a Brown sandy loam in the Brown soil zone developed on glacial till. S. comata was the dominant plant species at the site sampled. Samples of the Dark Brown soil Great Group were taken at the Pinhorn Ranch from NE 19-2-6 W4. This is a Dark Brown loam in the Brown soil zone developed on glacial till. S. spartea variety curtiseta was the dominant plant species at the site sampled. Samples of the Black soil Great Group were taken at the Streeter Basin from SW 27-13-1 W5. This is a Thin Black clay loam in the Black soil zone developed on glacial till. F. scabrella was the dominant plant species at the site sampled.



1. Onefour Substation - Brown Soil Zone
2. Pinhorn Ranch - Brown Soil Zone
3. Streeter Basin - Thin Black Soil Zone

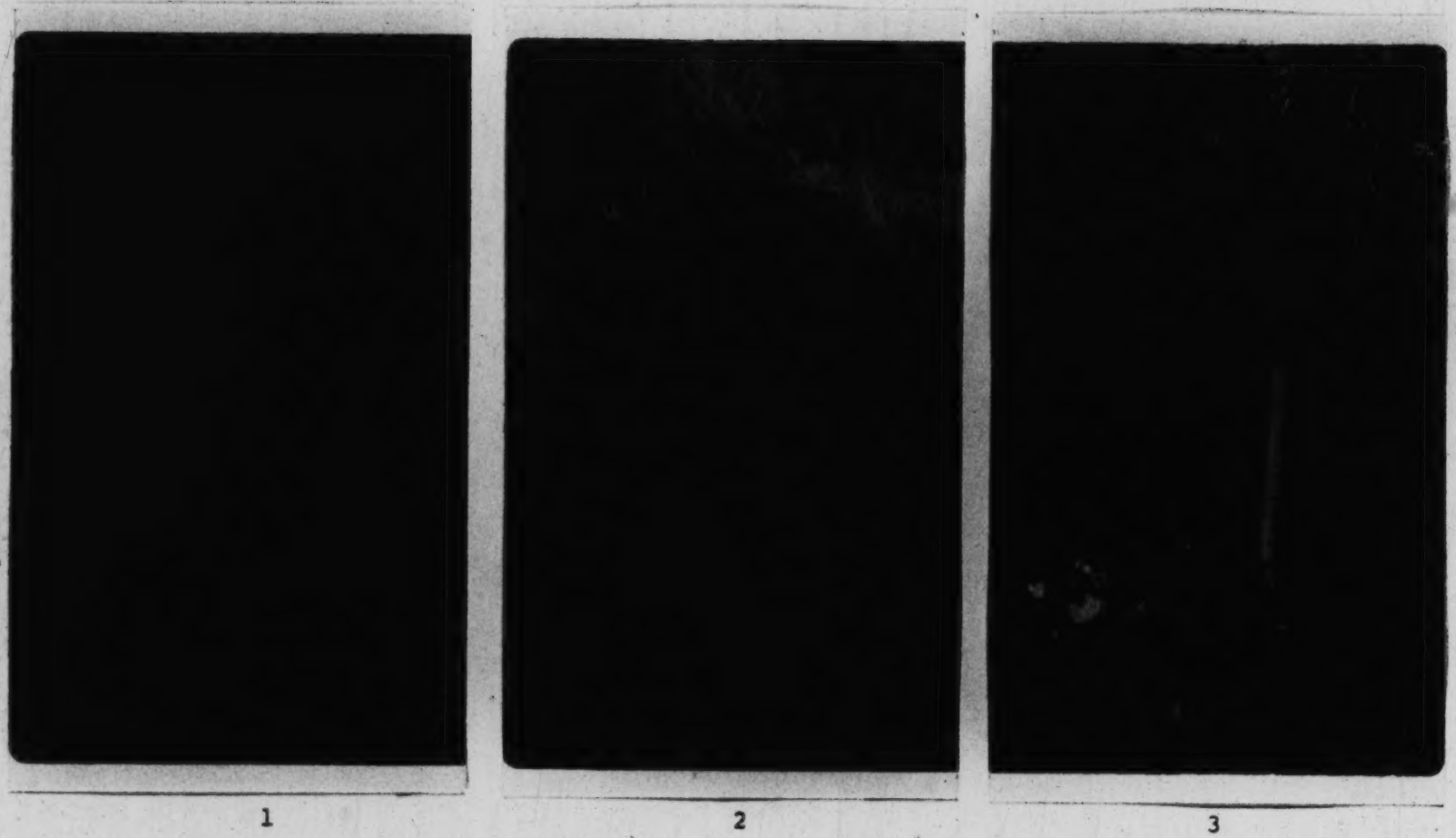
Figure 4. Sample sites.

Table 6. Profile Descriptions

Order Great Group	Horizon	Structure	Depth cm	Moist Color
Chernozemic Brown	Ah	Loose granular	0- 8	10 YR 5/4
	Upper Bt1	Strong columnar	8- 60	10 YR 4/4
	Lower Bt2	Weak columnar	60-117	10 YR 4/3
	Cca	Massive	117+	10 YR 6/2
Chernozemic Dark Brown	Ah	Loose granular	0- 10	10 YR 3/4
	Bt	Strong columnar	10- 55	10 YR 3/4
	Cca	Massive	55-110	10 YR 4/2
Chernozemic Black	LFH	Organic	2- 0	
	Ah	Strong prismatic	0- 8	10 YR 2/1
	Bt1	Strong prismatic	8- 26	10 YR 4/3
	Bt2	Strong subangular blocky	26- 57	10 YR 4/3
	Cca	Massive	57-100	10 YR 5/2

Table 7. General Description of Sites and Plant Communities Sampled

Site	Onefour Substation	Pinhorn Ranch	Streeter Basin
Legal Location	SE 16-2-4 W4	NE 19-2-6 W4	SW 27-13-1 W5
Dominant Species	Stipa comata	Stipa spartea variety curtiseta	Festuca scabrella
Common Name	Spear grass	Western porcupine grass	Rough fescue
Root Density	5,542 kgm.roots/ha.	6,438 kgm.roots/ha.	12,606 kgm.roots/ha.
Topography and Drainage	Flat, well drained	Flat, moderate drainage	Steeply sloping, east aspect, good drainage
Climate	Semi-arid	Semi-arid	Moist sub-humid
Soil Zone	Brown	Brown	Thin Black
Soil Texture	Sandy loam	Loam	Clay loam



Plates 1, 2 and 3. Profiles of Brown, Dark Brown and Thin Black soils which were sampled to obtain roots of Stipa comata, Stipa spartea variety curtiseta and Festuca scabrella, respectively.

Plate 4. Landscape, Brown Great Group, Onefour Substation, SE 16-2-4 W 4.

Plate 5. Stipa comata at above location.

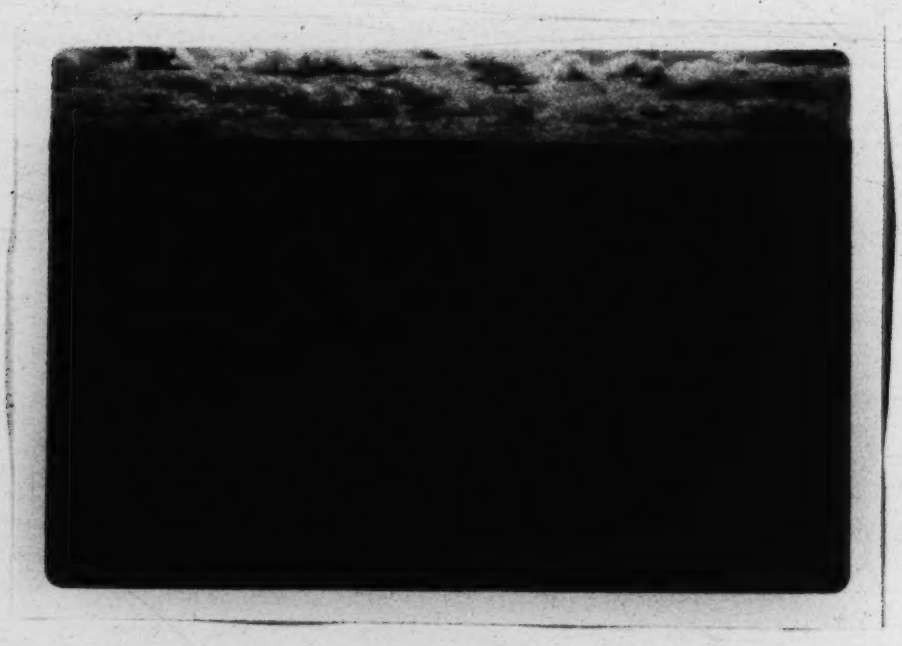


Plate 6. Landscape, Dark Brown Great Group, Pinhorn Ranch, NE 19-2-6 W4.

Plate 7. Stipa spartea variety curtiseta at above location.

Plate 8. Landscape, Black Great Group, Streeter Basin, SW 27-13-1 W5.

Plate 9. Festuca scabrella at above location.

Sampling Technique

Each site was sampled to a six inch depth with the surface growth cut away and discarded. The samples were washed by hand squeezing in a stream of water to carefully separate the root fibers from the mineral portion of the soil.

The roots were separately air dried and ground in a Wiley Mill, Model No. 3, to pass through a one millimeter screen.

Experimental Design

The main experiment was designed to facilitate the study of the chemistry of the fresh and decomposing root tissues and their rates of decomposition. To this end samples of three root species were individually incubated in duplicate at 28°C in quart (938 ml.) milk bottles for various lengths of time ranging from 2 weeks to 47 weeks. To each bottle, 20.000 g. of dried ground roots of one grass species was added with 200 ml. of distilled water. From each respective soil, a 10 ml. inocculum from 25 g. of soil shaken in 100 ml. of distilled water for 24 hours was added to each bottle. Air, scrubbed of CO_2 and airborn microbial spores, was passed through a glass tube reaching below the liquid

level in each bottle providing an oxygen supply and a method of slow mixing. Each incubation bottle was occasionally shaken by hand to prevent anaerobic conditions through settling of root particles. Respired gases and bubbled air was allowed to escape through a second hole in the stopper of each bottle (Plate 10).

Duplicate samples of each grass species were removed from the incubation system after 2, 4, 6, 8, 10, 12, 16, 20, 24, 37 and 47 weeks of incubation and prepared for analysis.

Analytical Techniques

A. CO_2 Respiration

The CO_2 respired by the microorganisms degrading the root material was monitored according to the methods described by Stotsky (1965). After bubbling into the incubation flask, the air and respired CO_2 was bubbled through an additional trap containing 1N NaOH (Plate 11). Atmospheric CO_2 was scrubbed before being bubbled into the incubation bottles by passing the air through 2N NaOH. In addition to removing CO_2 from the air supply, microbial spores and water was removed in the gas train (Plate 10) by glass wool and bubbling air through 2N H_2SO_4, respectively. Three samples of each grass species were monitored for a total of 91 days.

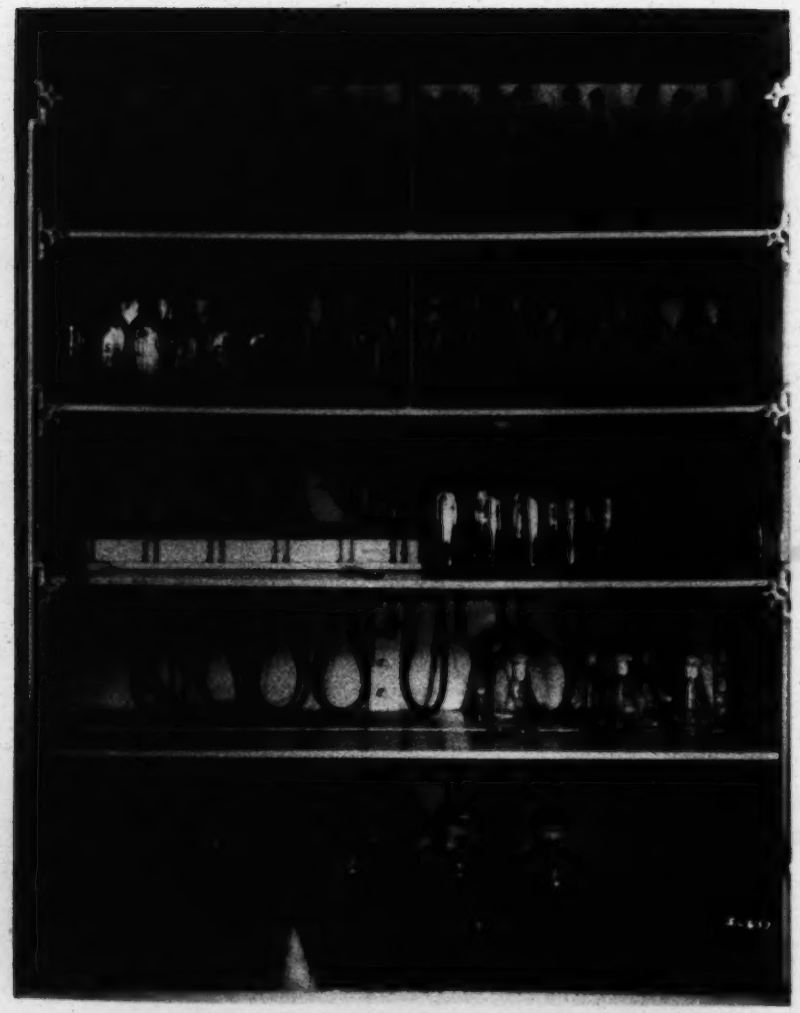


Plate 10. Incubation chamber.

Plate 11. Incubation bottle and CO_2 trap.

B. Sample Preparation

At selected incubation termination times, duplicate samples of each grass species were centrifuged for 5 minutes at 2,000 r.p.m. The liquid portion of each sample was filtered through a Whatman No. 4 filter paper and the pH taken with a Fisher Accumet Model 320 Research pH Meter. The filtrate (water soluble fraction) and the residue (non-water soluble fraction) of each sample were separately freeze-dried using a Virtis Freeze-drier and stored for later analysis.

C. Degree of Hydrolyzability

Residue samples were hydrolyzed in 6N HCl for 16 hours at 100°C (Mortensen, 1965). The hydrolysed material was passed through a glass filter. The filtrate was made up to 25 ml. with distilled water and the residue dried for 24 hours at 105°C.

D. Elemental Analyses

1. Total Carbon

Total carbon determinations were done on both the water soluble and residue fractions by dry combustion at 900°C for 15 minutes in a stream of O_2. The evolved CO_2 was collected in Ascarite. A platinum chloride catalyst was used to ensure complete conversion of organic carbon to CO_2 (Allison, Bollen and Moodie, 1965).

2. Total Nitrogen

The total nitrogen content of soil and residue samples was determined by the regular macro-Kjeldahl method described by Bremner (1965). The total nitrogen content of the water soluble fraction was determined by the semimicro-Kjeldahl method described by Bremner (1965).

3. Total Hydrogen

Total hydrogen was determined with a Coleman Carbon-Hydrogen Analyser, Model No. 33, by the Microanalytical Laboratory, Department of Chemistry, University of Alberta.

4. Total Phosphorus

Total phosphorus was determined using the perchloric acid digestion followed by colorimetric determination with sulfomolybdic acid as described by Olsen and Dean (1965).

E. NH_4 and NO_3

NH_4 and NO_3-N determinations were done by the steam distillation method described by Bremner (1965).

F. Functional Groups

1. Acidic Functional Groups

The total and carboxyl acidity of samples were

determined by the procedures described by Schnitzer and Gupta (1965). Phenolic acidity was evaluated by the difference between values for total and carboxyl acidity.

2. Methoxyl Groups

The methoxyl content of each residue sample was determined by the Zeisel semimicro method described by Clark (1929). An additional test tube of liquid bromine was utilized to ensure complete trapping of CH_3I carried in the stream of CO_2.

G. Fundamental Polymers

1. Lignin

The method described by Czerkawski (1967) was used to determine the residue lignin content. Soxhlet extraction with ethanol and benzene extractants followed by refluxing with 1N H_2SO_4 removed all other plant constituents from the residue samples. The remaining lignin was dried, weighed and corrected for ash content.

2. Carbohydrates

The procedure described by Deriaz (1961) was used for the determination of residue soluble and structural carbohydrates. Samples were successively extracted with diethyl ether, 0.5 percent ammonium oxalate solution, 1N H_2SO_4 and 72 percent H_2SO_4. Soluble carbohydrates in the

ammonium oxalate solution were determined by anthrone. The hydrolysed pentosans and hexosans in the combined 1N H_2SO_4 and 72 percent H_2SO_4 extracts were determined with aniline acetate and chromotropic acid, respectively.

3. Amino Acids

Residue amino acids were extracted in 6N HCl at 100°C for 16 hours. The hydrolyzate was cleansed of impurities on Doxex 50 (x-8) cation exchange columns. Total amino acids in the residues were determined by the Moore and Stein (1954) colorimetric method using ninhydrin. Absorbance was measured at 570 mμ on a Bausch and Lamb Spectronic 20 colorimeter. The relative molar ratios of residue amino acids, acylated n-butyl esters with trifluoroacetic acid, were determined using the gas chromatographic method of Roach and Gehrke (1969). A $2x10^{-6}$ mole ornithine internal standard was used in every sample.

H. Infrared, Ultraviolet and Visible Spectra

A Perkin Elmer Model 457 was utilized to produce the infrared spectra of the residue and water soluble samples. Each sample was pressed to a pellet in KBr at 18,000 p.s.i. pressure for five minutes under vacuum. Freeze drying each sample reduced moisture interference during printing of the infrared spectra.

The ultraviolet and visible spectra of the water soluble fraction was produced using a Cary 20 Spectrophotometer over a 200 to 360 and 360 to 600 nanometer range, respectively. A distilled water medium was used.

I. Soil Particle Size Distribution

The pipette procedure described by Day (1965) was used to determine particle size distribution of the Ah horizons of the soils sampled.

J. Ash Content

The ash content of the samples was determined by ignition for four hours at 550°C.

K. Photomicrographs

Photomicrographs of raw and decomposing root particles were taken with Kodak Versipan film. The root particles were mounted in a well slide using a water medium, a cover slip, a 10 power ocular lens and 10 power objective lens of Weiss microscope.

RESULTS

Root Decomposition

The three grass species decomposed rapidly during the initial 10 weeks of incubation; Stipa comata and Festuca scabrella each losing 22 percent of their original root weight and Stipa spartea variety curtiseta losing 24 percent (Figure 5). Greater differences in the amount of root weight lost by each species became apparent in the remaining 37 weeks of incubation; S. spartea having lost a total of 40 percent; F. scabrella 35 percent and S. comata 31 percent when incubation was terminated. Loss of organic material from the root residues was not accompanied by a concomitant increase in organic material in the water soluble fraction (Table 8).

Table 8. Weight of Water Soluble Organic Materials at Various Sampling Times, from Three Grass Species Decomposing at 28°C

Decomposition Time (weeks)	Weight of Water Soluble Organic Material (g)		
	F. scabrella	S. spartea	S. comata
2	.09	.11	.22
8	.13	.11	.18
37	.10	.06	.10

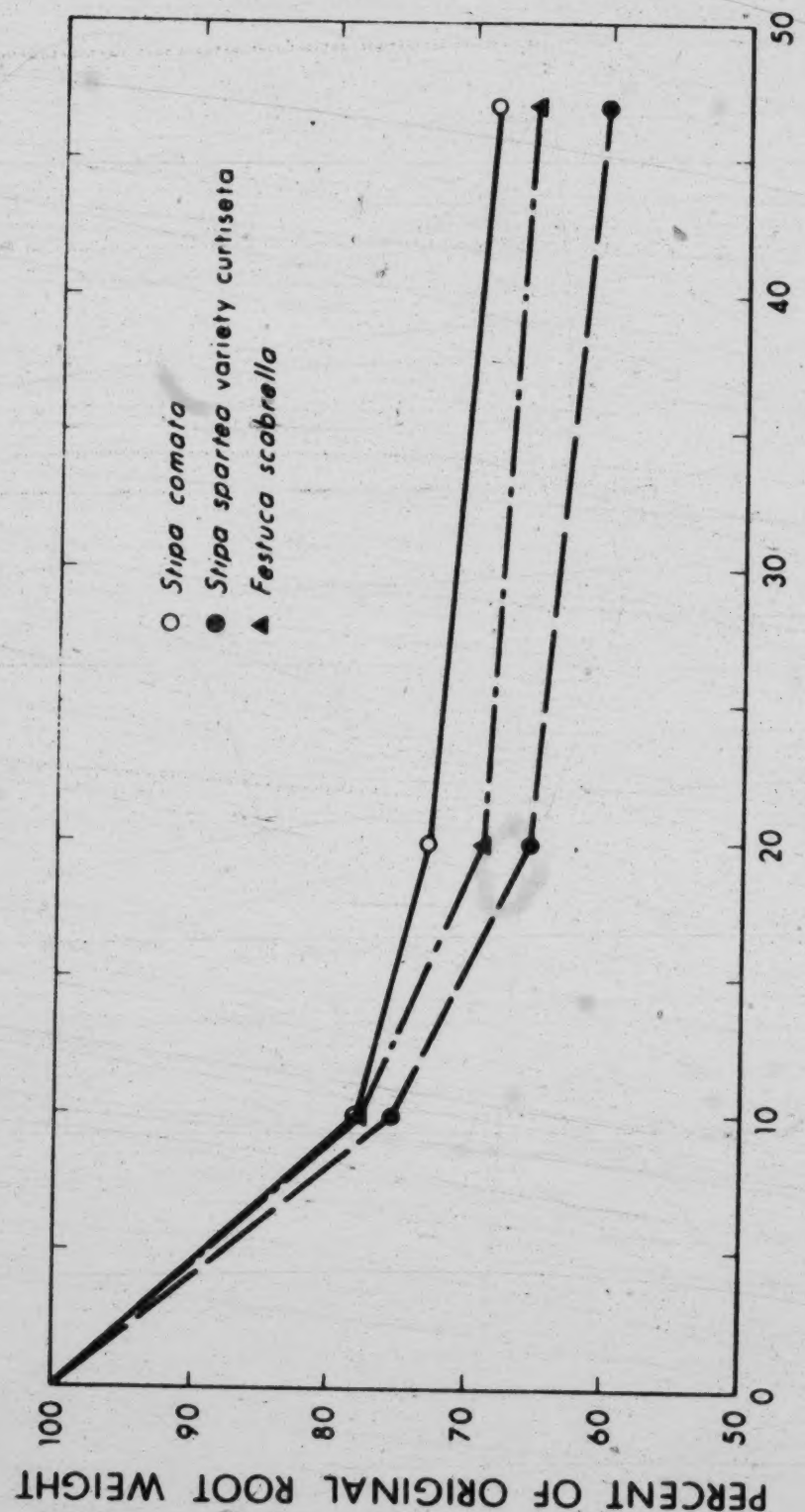


Figure 5. Decomposition of roots of three grass species incubated at 28°C.

The raw root particles of each species, when viewed microscopically, consisted of defined xylem, exodermis and endodermis tissues. Grinding of the air dried roots before incubation caused a small amount of maceration of the exodermis (Plates 12, 14 and 16). The physical decomposition of each species was a loss of the well defined internal root structures (Plates 13, 15 and 17). The root particles of S. spartea and S. comata, after 47 weeks, retained only the skeleton-like cell walls of the endodermis. The F. scabrella particles retained much of the internal materials but in a much less defined form then were found in the undecomposed tissues.

CO_2 Evolution

Microbial activity was monitored during the first 91 days of incubation by measuring CO_2 evolution. The respired carbon from the residues of F. scabrella, S. spartea and S. comata amounted to 2.4, 2.1 and 2.4 g., respectively, and accounted for 27 percent, 27.5 percent and 29 percent, respectively, of the raw root carbon content (Table 9 and Figure 6).

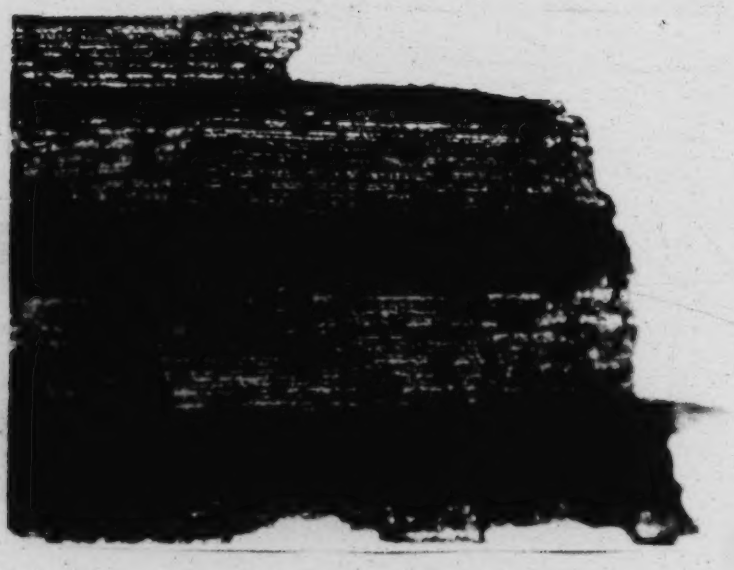


Plate 12. Raw root (x 100) of Festuca scabrella.

Plate 13. Festuca scabrella root residue (x 100) after 47 weeks incubation at 28°C.

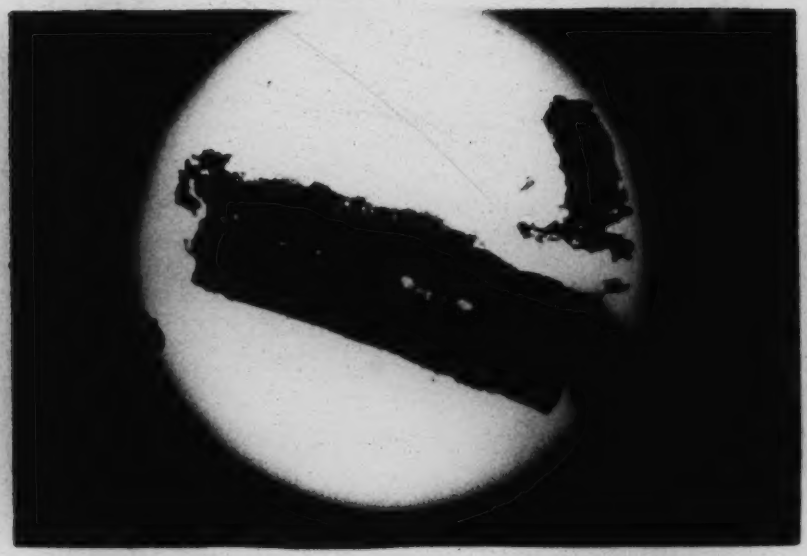


Plate 14. Raw root (x 100) of Stipa spartea variety curtiseta.

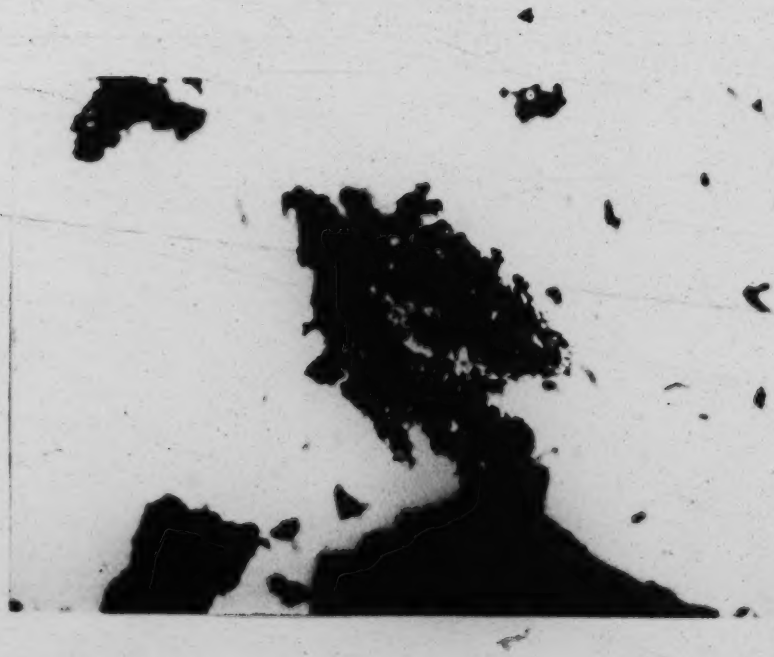


Plate 15. Stipa spartea variety curtiseta root residue (x 100) after 47 weeks incubation at 28°C.

Plate 16. Raw root (x 100) of Stipa comata.

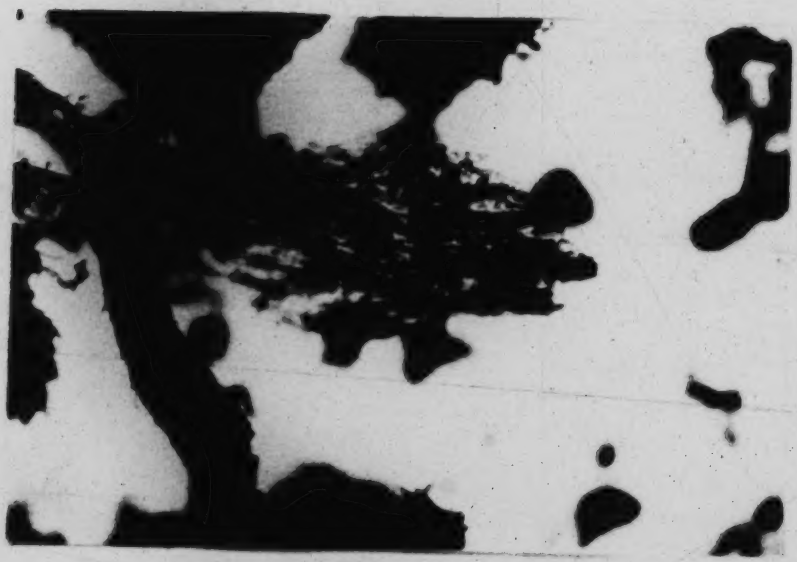


Plate 17. Stipa comata root residue (x 100) after 47 weeks incubation at 28°C.

Table 9. Cumulative Carbon Respired from the Roots of Three Grass Species Incubated at 28°C

Decomposition Time (days)	Cumulative Respired CO_2 - C (mg)		
	F. scabrella	S. spartea	S. comata
.5	38	35	35
1	180	124	96
2	247	138	110
3	303	197	159
5	472	271	259
8	598	379	399
15	799	572	599
22	959	755	757
29	1,094	874	959
36	1,216	996	1,152
43	1,378	1,129	1,337
50	1,559	1,310	1,516
57	1,695	1,437	1,659
64	1,856	1,626	1,837
71	1,994	1,790	2,020
78	2,199	1,973	2,199
91	2,359	2,139	2,377

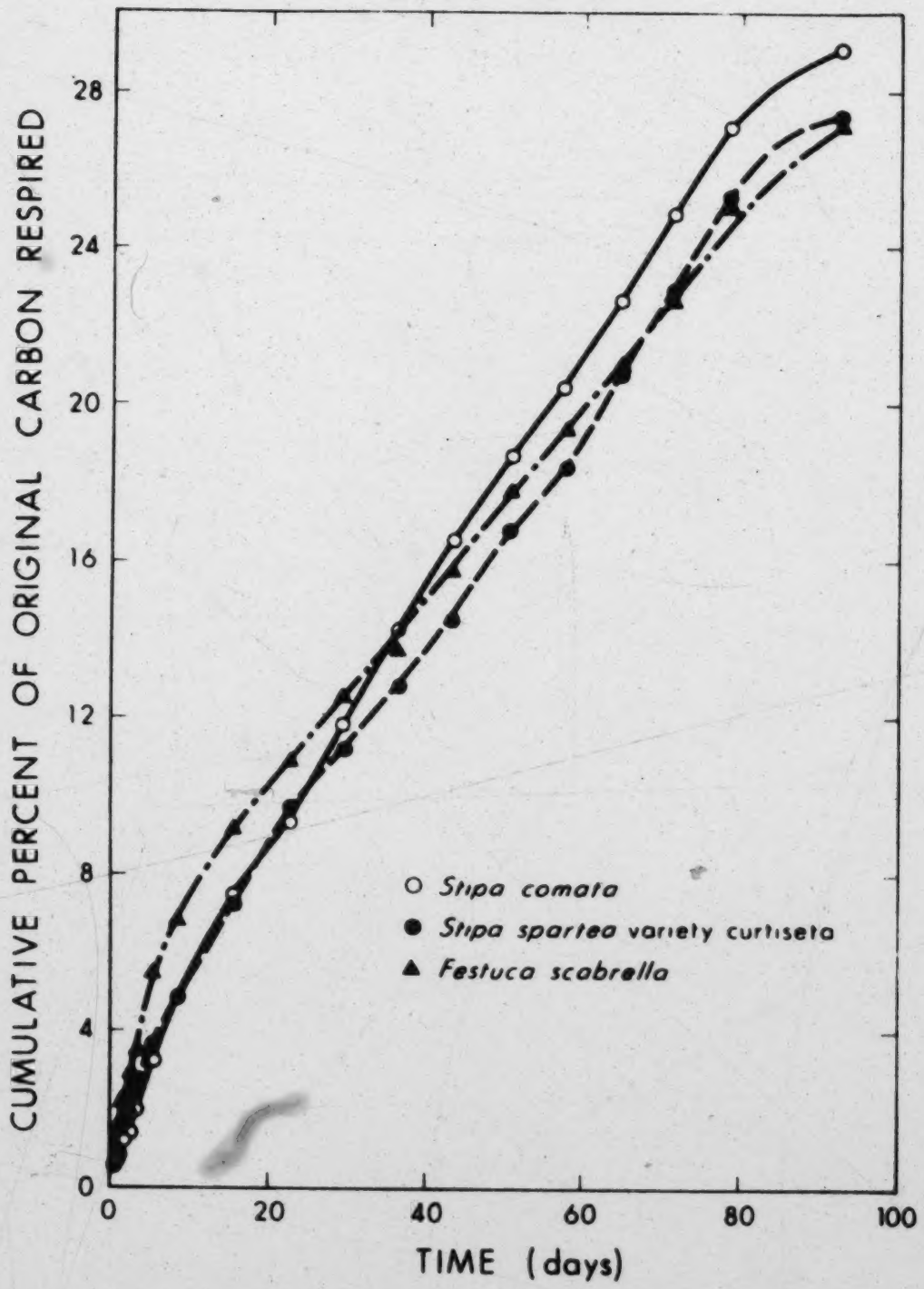


Figure 6. Cumulative percent of original carbon respired during decomposition of roots of three grass species for 13 weeks at 28°C.

Elemental Distribution

The raw roots and residues after 10, 20 and 47 weeks of decomposition of all three species were analyzed for carbon, nitrogen, hydrogen and phosphorus content (Table 10).

Carbon content in the residues of all three species rapidly declined in the initial 10 weeks of incubation (Figure 7). A moderate loss occurred in the 10 to 20 week interval followed by little or no loss in the final 27 weeks of incubation. S. comata and F. scabrella lost 32 percent of their original root carbon while S. spartea lost 30 percent over the 47 week incubation.

Residue nitrogen content decreased in all three species during the initial 20 weeks of decomposition (Figure 7). The residue nitrogen content of S. spartea gradually increased in the final 27 weeks of incubation to 80 percent of its original value.

The absolute weight of carbon was of the same order for all three species; the order of nitrogen was not. S. comata and S. spartea contained greater amounts of nitrogen in the raw root tissues (Table 10). The C/N ratio of the raw roots of S. comata and S. spartea was 18/1 and 22/1, respectively; the raw roots of F. scabrella having a higher ratio at 35/1 (Figure 8). The C/N ratio of the residues of all three species remained relatively constant during decomposition.

Table 10. Elemental Content of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Species	Element (g)	Grams of Element in Total Residue* Decomposition Time (weeks)			
		Raw Roots	10	20	47
F. scabrella	C	8.78	6.61	5.99	5.96
	N	.25	.17	.16	.16
	H	1.18	.90	.82	.80
	P	.04	.02	.02	.02
S. spartea	C	7.81	6.10	5.40	5.61
	N	.36	.31	.26	.29
	H	.93	.78	.75	.68
	P	.04	.03	.03	.03
S. comata	C	8.12	6.40	5.57	5.43
	N	.45	.37	.37	.38
	H	.97	.81	.68	.53
	P	.04	.04	.03	.03

* Originally 20.000 g of roots added to incubation bottle. For total residue weights see Appendix 1.

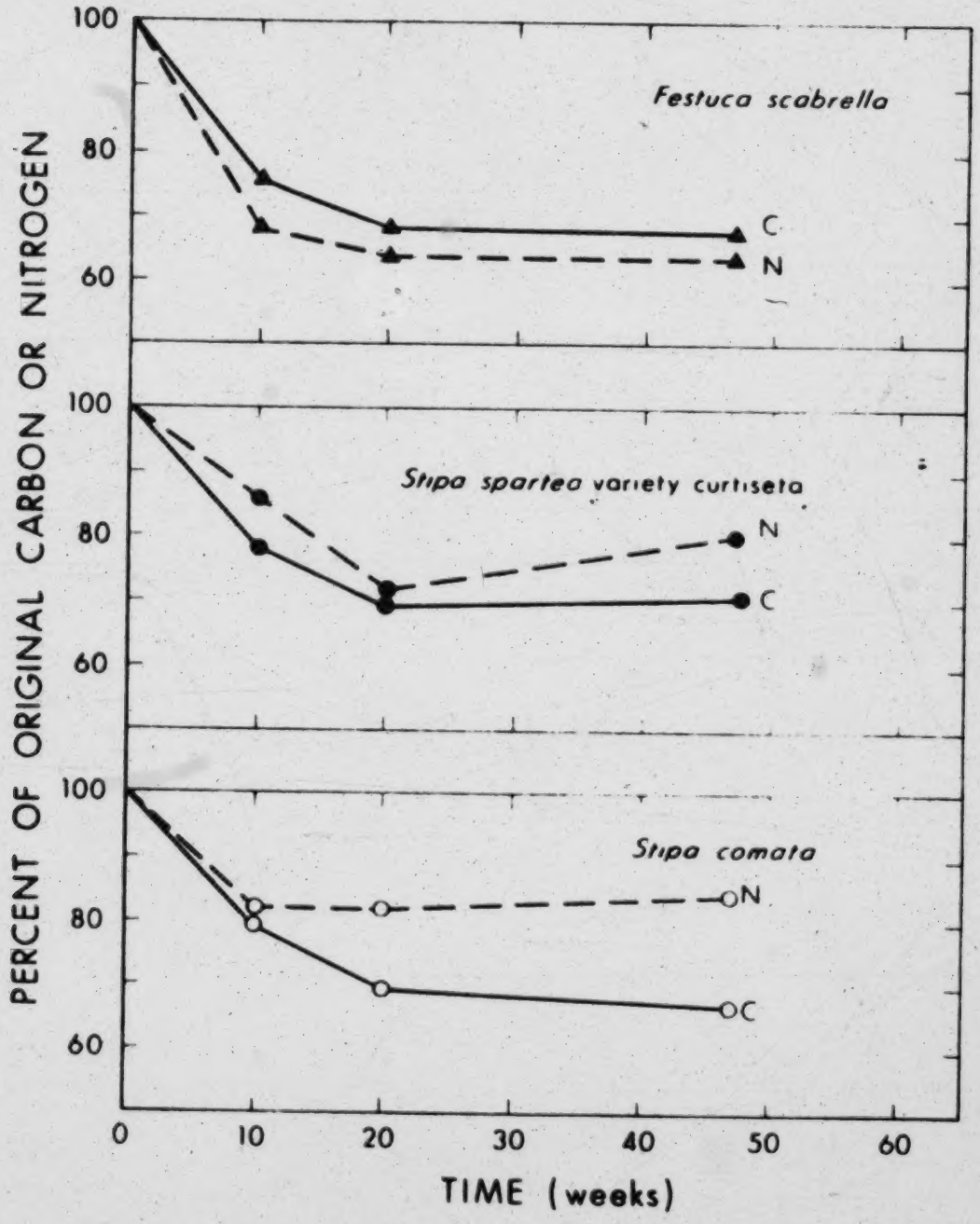


Figure 7. Carbon or nitrogen remaining in raw and decomposing roots of three grass species decomposing at 28°C.

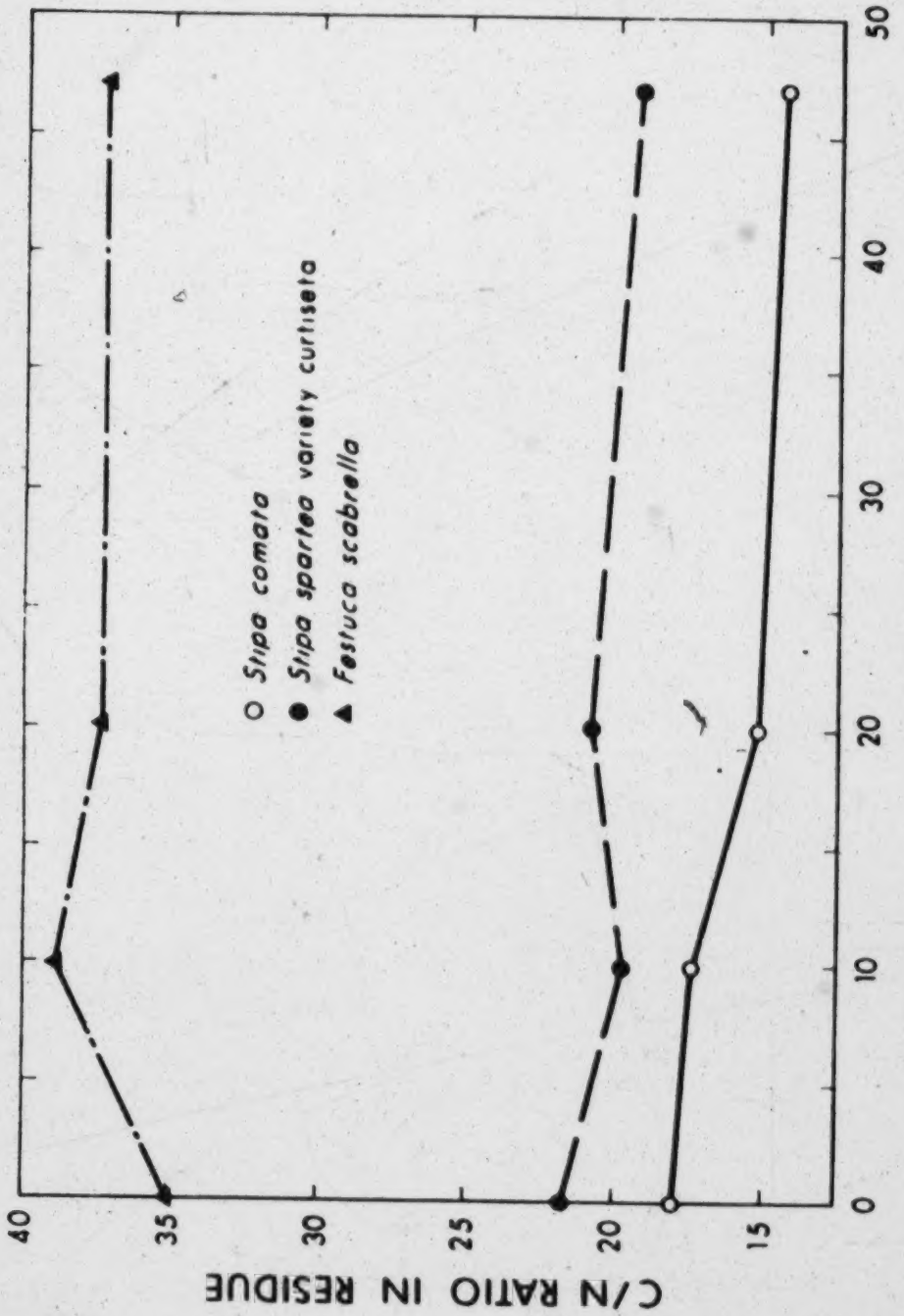


Figure 8. C/N ratio of raw and decomposing roots of three grass species decomposing at 28°C.

Residue total hydrogen content continuously decreased in all three species during decomposition (Figure 9). The residues of S. comata lost the greatest amount of total hydrogen with a near linear rate of decrease over 47 weeks of incubation.

A greater loss of residue hydrogen relative to losses of residue carbon resulted in an increasing C/H ratio in the residues of S. spartea and S. comata during incubation (Figure 10). The C/H ratio of the residues of F. scabrella remained unchanged.

Residue phosphorus, during the initial 10 weeks of incubation, decreased in all three species (Figure 11). The residue phosphorus content of all three species slowly increased during the final 27 weeks of incubation. Overall loss of residue phosphorus in all three species could not be accounted for in the water soluble fraction of each species and may be a procedural error.

Accurate weights of all the freeze dried water soluble fractions were not determined; the few samples of each species which were weighed contained .22 g or less material. In all cases, the amount of carbon, nitrogen, hydrogen and phosphorus in the water soluble fraction was less than the losses of these elements from the decomposing residues (Table 11). A small amount of the water soluble nitrogen was accounted for as ammonium and nitrate nitrogen (Table 12).

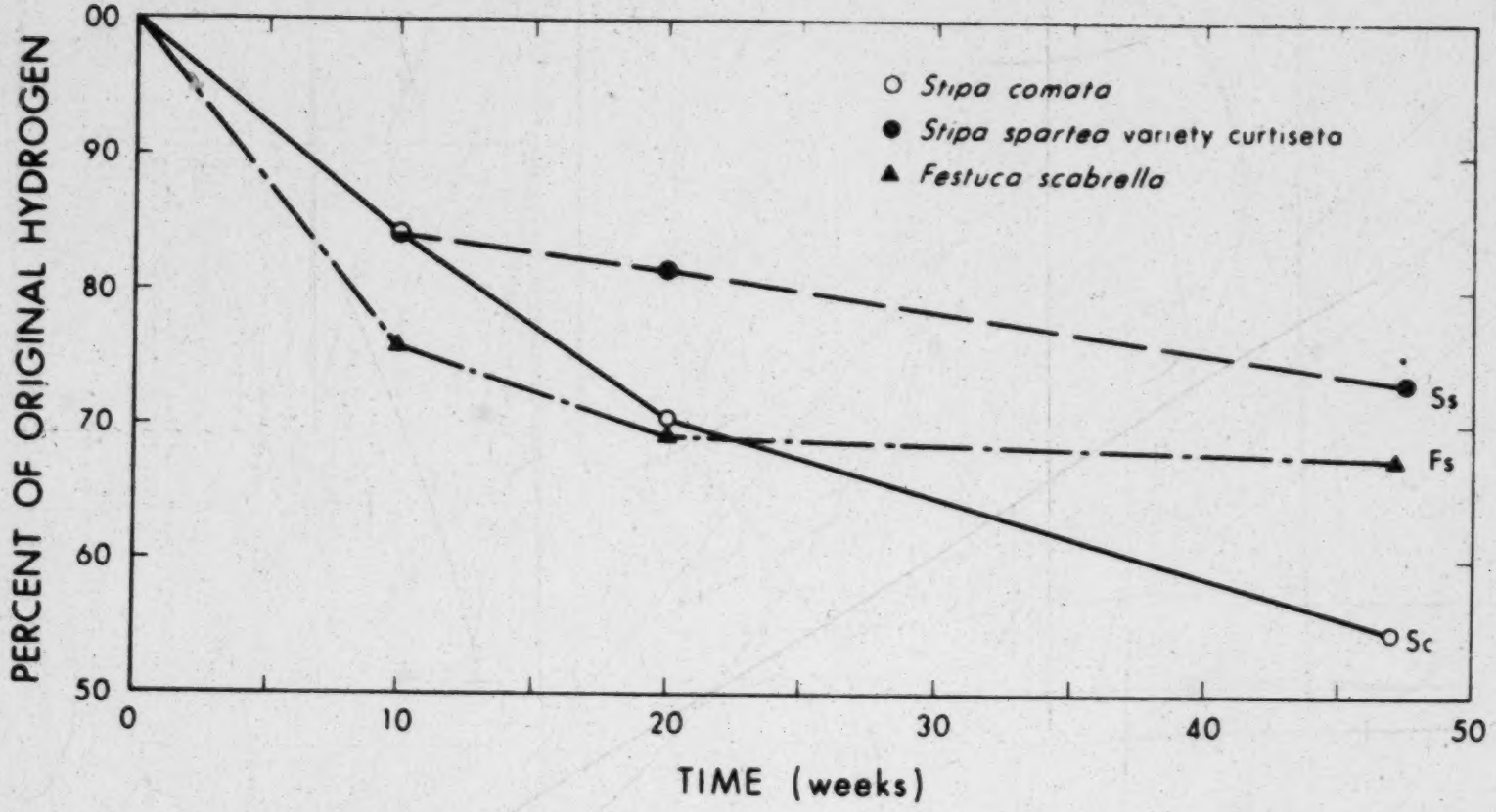


Figure 9. Hydrogen content of raw and decomposing roots of three grass species decomposing at 28°C.

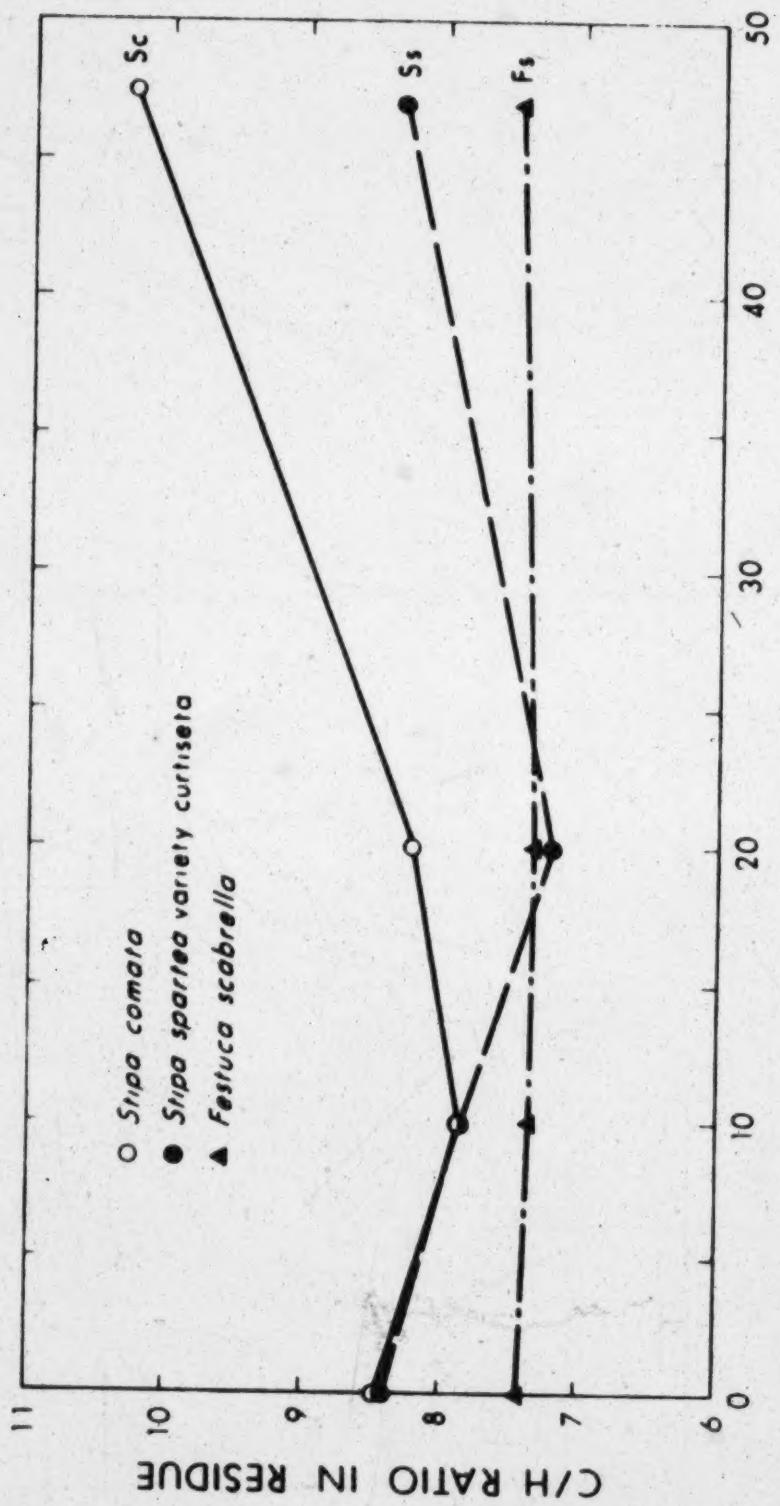


Figure 10. C/H ratio of raw and decomposing roots of three grass species decomposing at 28°C.

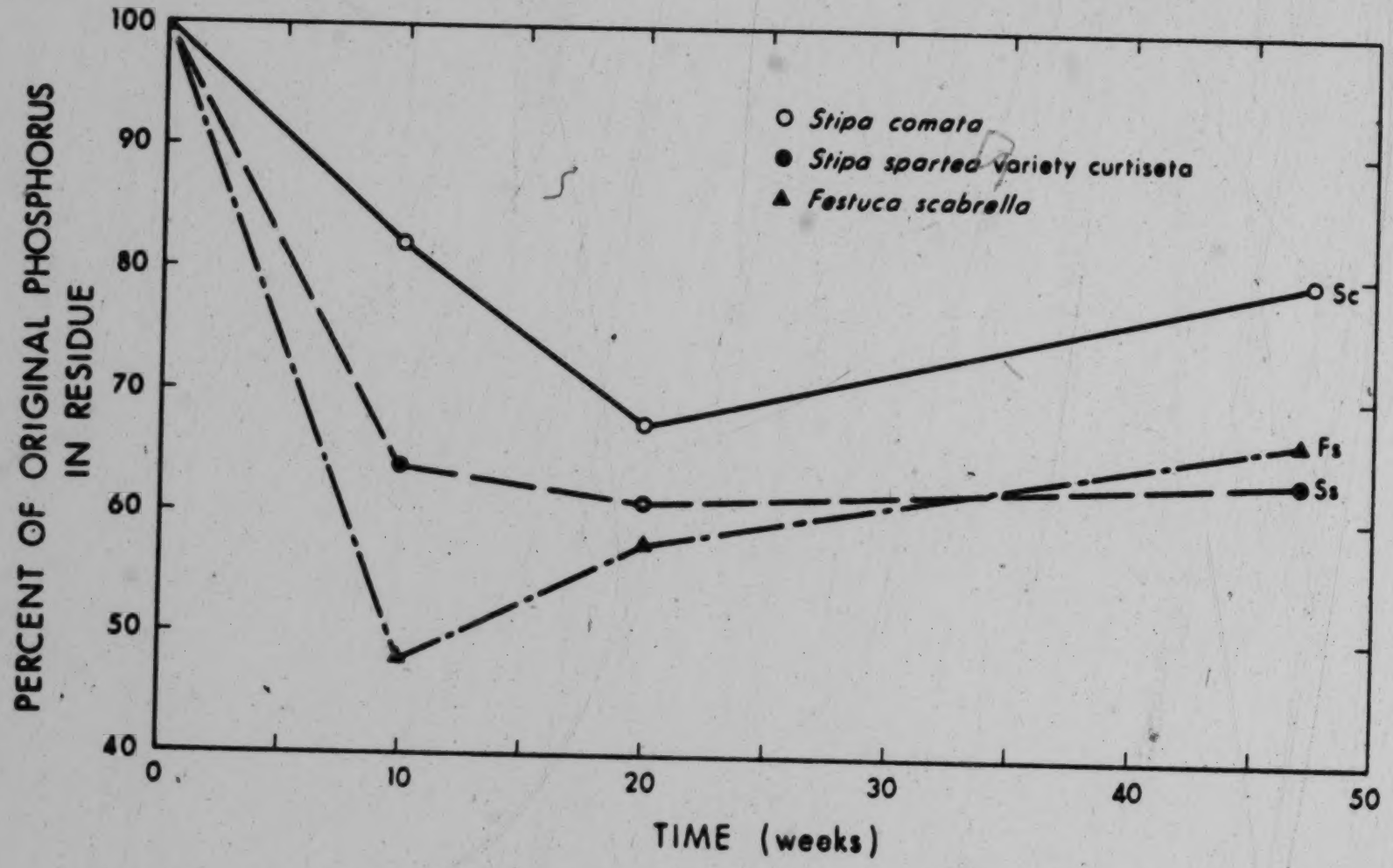


Figure 11. Phosphorus content of raw and decomposing roots of three grass species decomposing at 28°C.

Table 11. Elemental Content of the Water Soluble Degradation Products of Three Grass Species Decomposing at 28°C

Species	Element (%)	Elemental Content (% dry wt.)* Decomposition Time (weeks)			
		Raw Roots	10	20	47
P. scabrella	C	33	20	25	24
	H	5	3	4	3
	P	5	2	2	3
S. spartea	C	30	21	22	25
	H	5	4	3	4
	P	6	5	7	5
S. comata	C	30	25	27	23
	H	5	3	4	3
	P	5	1	1	2

* For total dry weights see Table 8.

Table 12. Nitrogen Content of the Water Soluble Degradation Products of Three Grass Species Incubated at 28°C

Species	Form of N	% N	
		6 Weeks Decomposition	37 Weeks Decomposition
F. scabrella	Total	2.4	2.1
	NH_4^+ - N	.07	.06
	NO_3^- - N	.06	.02
S. spartea	Total	3.2	1.8
	NH_4^+ - N	.19	.8
	NO_3^- - N	.6	.15
S. comata	Total	2.6	2.1
	NH_4^+ - N	.11	.04
	NO_3^- - N	.03	.09

Constituent Distribution

Residue percent lignin increased in all three species during decomposition possibly due to a more rapid decomposition of other root constituents (Table 13). Percentage increases in lignin content of the residues of F. scabrella, S. spartea and S. comata were 21 percent, 31 percent and 32 percent, respectively. The lignin constituent did degrade in all three species. Percentage of original lignin lost from the residues of F. scabrella, S. spartea and S. comata was 21 percent, 21 percent and 10 percent, respectively.

Raw root and residue percent nonhydrolyzable material remained between 51 percent and 61 percent in all three species during incubation (Table 14). Percentage loss of nonhydrolyzable material in the residues of F. scabrella, S. spartea and S. comata was 31 percent, 42 percent and 25 percent, respectively after 47 weeks. The results demonstrate degradation even of organic tissues which are resistant to acid hydrolysis. The greatest degradation occurred in the roots and residues of S. spartea.

The majority of raw and decomposing root carbohydrates were in the form of structural carbohydrates (Table 15). The decomposing roots of S. comata lost the greatest amount of structural carbohydrates, 61 percent, while the tissues of F. scabrella and S. spartea lost 43 percent and 39 percent, respectively (Figure 12).

Table 13. Lignin Content of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Percent of Residue Weight			Percent of Original Lignin Remaining		
	F.scabrella	S.spartea	S.comata	F.scabrella	S.spartea	S.comata
Raw roots	29	38	37	100	100	100
10	28	44	52	76	86	100
20	31	46	47	73	78	97
47	35	50	49	79	79	90
Percent change	+21	+31	+32	-21	-21	-10

Table 14. Nonhydrolyzable Material Content of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Percent of Residue Weight			Percent of Original Nonhydrolyzable Material		
	F.scabrella	S.spartea	S.comata	F.scabrella	S.spartea	S.comata
Raw roots	51	60	57	100	100	100
4	52	64	55	88	84	89
12	52	N.D.	60	71	N.D.	84
20	50	54	56	70	58	76
47	55	58	60	69	58	72
Percent change				-31	-42	-28

Table 15. Soluble and Structural Carbohydrate Content of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Percent of Residue Weight					
	F. scabrella		S. spartea		S. comata	
	Structural	Soluble	Structural	Soluble	Structural	Soluble
Raw roots	43.2	.3	29.8	.2	32.7	.1
10	34.9	.1	23.8	.1	20.7	.1
20	29.5	.1	21.5	.1	20.0	.1
47	24.6	.1	17.9	.1	12.7	.1
Percent change	-43.1		-39.3		-61.2	

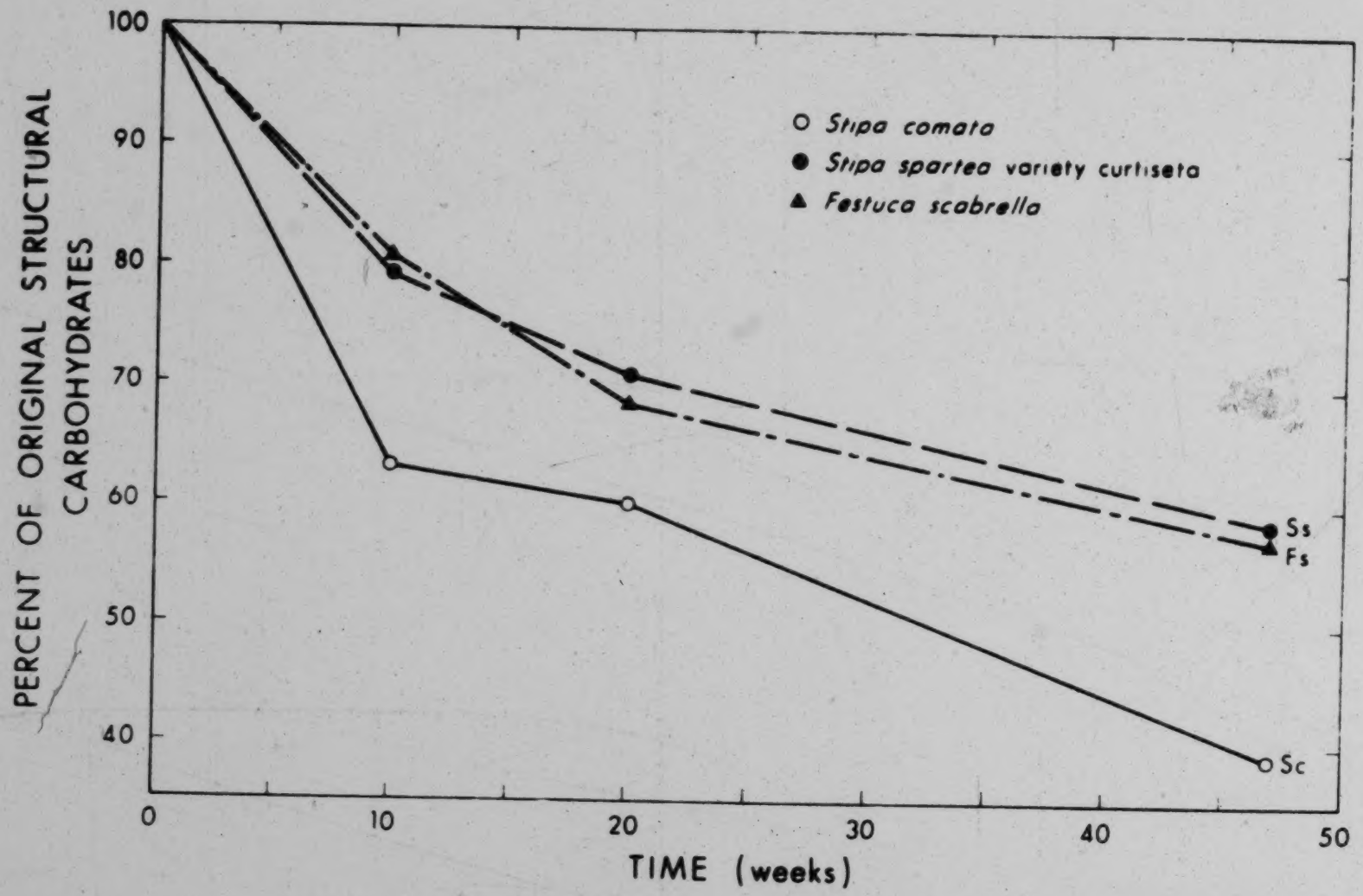


Figure 12. Structural carbohydrate content of raw and decomposing roots of three grass species decomposing at 28°C.

The decomposing roots of S. comata lost the greatest amount of structural carbohydrates in 47 weeks, but lost the least amount of lignin. The residues of F. scabrella and S. spartea each lost about 40 percent of their structural carbohydrates and 21 percent of their lignin. S. comata lignin loss amounted to only 10 percent. In all three species, the structural carbohydrates degraded and loss exceeded the loss of lignin from the residues.

The amino acid percent distribution and total content was assayed in the residues of all three species after 4 and 37 weeks of incubation (Table 16). Amino acid changes in types and amount were evaluated as an indicator of changes in microbial enzymes. Changes in amino acid percent distribution was expressed as an absolute range or sum of the range of percentage distribution of each amino acid for each grass species over the 33 week interval evaluated. The largest absolute range was 21.1 percent in the residues of S. spartea. This figure may be indicative of the greatest degree of enzyme alteration in the microorganisms degrading S. spartea tissues. Enzyme alterations are conducive to a greater versatility of enzymes to degrade different substrates. The weight of total amino acids in the residues of S. spartea also increased from an initially low value of .26 g to .74 g. This may be indicative of a greater weight of enzymes degrading the residues of S. spartea. The greater enzyme weight may be conducive to a greater capacity to degrade the given substrate. Residues of F. scabrella, with

Table 16. Amino Acid Percent Distribution and Total Content in Raw and Decomposing Roots of Three Grass Species Decomposing at 28°C

	Percent Distribution					
	F. scabrella		S. spartea		S. comata	
Decomposition Time (weeks)	4	37	4	37	4	37
Amino Acid						
Alanine	15.3	15.9	11.7	8.8	12.1	11.4
Valine	8.3	7.5	8.2	5.9	9.5	10.5
Glycine	14.0	13.7	17.6	13.9	12.4	9.7
Isoleucine	6.5	6.5	6.3	5.6	8.1	7.6
Leucine	10.1	12.8	11.6	11.4	14.0	14.0
Proline	6.8	7.0	6.0	8.1	6.4	9.6
Threonine	4.3	5.2	4.9	4.8	4.8	6.2
Serine	5.7	5.2	5.8	5.1	4.9	5.2
Phenylalanine	3.6	4.9	4.4	6.3	5.2	6.9
Aspartic acid	9.3	9.0	8.2	9.1	9.2	7.9
Glutamic acid	8.3	6.1	7.6	12.8	6.3	5.1
Lysine	8.0	6.3	7.8	8.2	6.8	5.9
Absolute Range*	10.5%		21.1%		15.2%	
Total Weight (g)	.28	1.14	.26	.74	1.04	.42
Percent Change	+307		+185		-60	

* Calculated by: Summation |% Amino Acid (Week 4 - Week 37)|.

the lowest absolute range of 10.5 percent, also increased their total amino acid weight from .28 g to 1.14 g. Residues of S. comata, with the intermediate absolute range of 15.2 percent, was the only species to decrease in total amino acid total weight from 1.04 g to .42 g. The increases in the total amino acid content in the residues of all three species may be a result of microbial synthesis of amino acids. Flaig (1970) reported addition of easily decomposable plant material to soil increasing the amount of amino acids through microbial synthesis.

Leucine, glycine and alanine were generally present in higher amounts in the residues of all three species at both assay times. Flaig (1970) reported similar results with soil hydrolyzates.

Functional Groups

Many authors have successfully utilized decreasing numbers of methoxyl groups in degrading organic tissues as an index of humification. The numbers of methoxyl groups decreased only in the residues of F. scabrella and actually increased in the residues of S. spartea and S. comata (Table 17).

The loss of a methoxyl group during decomposition of organic tissues is an oxidative process with the production of one carboxyl group per oxidized methoxyl group (Oglesby, Christman and Driver, 1967). The carboxyl hydrogen content

Table 17. Methoxyl Group Content of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Methoxyl Group Content (mg)		
	F. scabrella	S. spartea	S. comata
Raw roots	96	72	59
2	64	117	129
4	51	108	133
6	50	107	86
8	45	95	67
10	78	57	68
12	52	54	81
16	55	53	90
20	58	43	72
24	65	84	72

of the residues of all three species decreased during incubation (Table 18). A situation of reducing conditions, rather than oxidizing conditions, may have existed in the incubating system. Carboxyl groups may have been reduced to methoxyl groups rather than methoxyl groups oxidized to carboxyl groups.

The total acid hydrogen in the residues of F. scabrella and S. spartea decreased from an initial 34 mg and 33 mg, respectively, to 18 mg and 26 mg, respectively (Table 18). Total acid hydrogen in the residues of S. comata increased from 22 mg to 25 mg in the incubation period. The residue total acid hydrogen so exceeded the carboxyl hydrogen in all three species that the phenolic acid hydrogen (by difference) followed the trend of total acid hydrogen (Table 18).

The pH of the distilled water medium utilized to harbour the microorganisms and decomposing roots, remained between 8.3 and 7.5 for all three species during incubation (Table 18).

Spectroscopic Analysis

The infrared, ultraviolet and visible spectra of the water soluble material for all three species were studied to gain information concerning the chemical structure of the materials.

Table 18. Total Acid, Carboxyl and Phenolic Acid Hydrogen Content and pH of Water Medium of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Species	Form of Hydrogen (mg)	Total, Carboxyl and Phenolic Hydrogen (mg) and Medium pH: Decomposition Time (weeks) Raw Roots	10	20	47
F. scabrella	Total acid	34	21	23	18
	Carboxyl	7	3	3	3
	Phenolic	27	18	20	15
	pH	8.2	8.1	8.1	
S. spartea	Total acid	33	29	26	26
	Carboxyl	8	3	4	4
	Phenolic	25	26	22	22
	pH	8.0	7.6	7.9	
S. comata	Total acid	22	26	22	25
	Carboxyl	5	3	3	3
	Phenolic	17	23	19	22
	pH	8.0	8.3	8.3	

The infrared spectra of the water soluble fraction for all three species suggest the fraction to contain many different types of bonds which change in type and number during decomposition of the root tissues (Figures 13, 14 and 15). The three species had similar spectra and only slight modifications in the spectra occurred during the decomposition period. Each species gradually lost a peak at 2,900 cm^{-1} (aliphatic C-H) indicative of decomposition of aliphatic molecules and groups. The spectra of each species gradually shifted a peak at 1,620 cm^{-1} (C=C stretch) to 1,600 cm^{-1} and gradually shifted a peak at 1,410 cm^{-1} to 1,380 cm^{-1} (salts of COO^-). The formation of a 1,380 cm^{-1} peak may have been a result of cellular disruption releasing cations to react with carboxyl groups to form respective salts. The spectra of each species indicated the possibility of unsaturated carbon bonding in the water soluble fraction. The water soluble material from the decomposing residues of F. scabrella completely lost a peak at 1,720 cm^{-1} (COOH) after 10 weeks of incubation.

The infrared spectra of the decomposing roots after 2 and after 47 weeks of decomposition in all three species indicated little or no change in the types and intensity of bonding in the residue materials (Figure 16).

The ultraviolet spectra of the water soluble fractions of each species had no definite peaks useful in structural determinations (Figures 17, 18 and 19). In

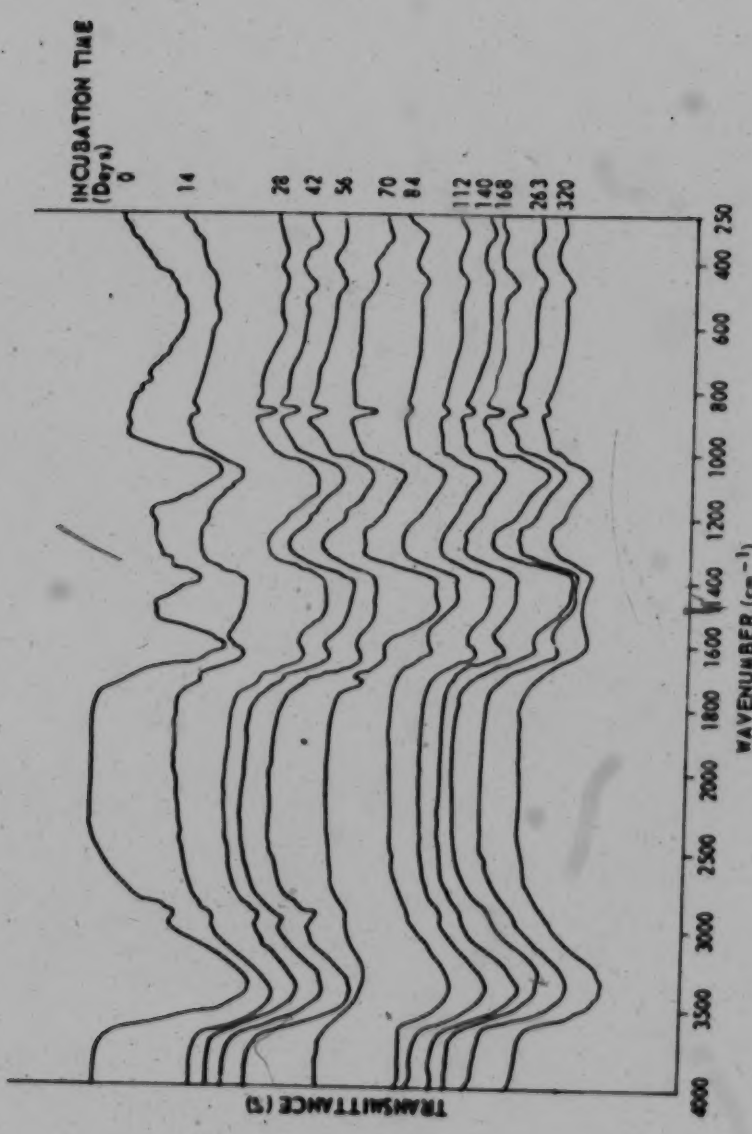


Figure 13. Infrared spectra of the water soluble fraction of raw and decomposing roots of Festuca scabrella incubated at 28°C.

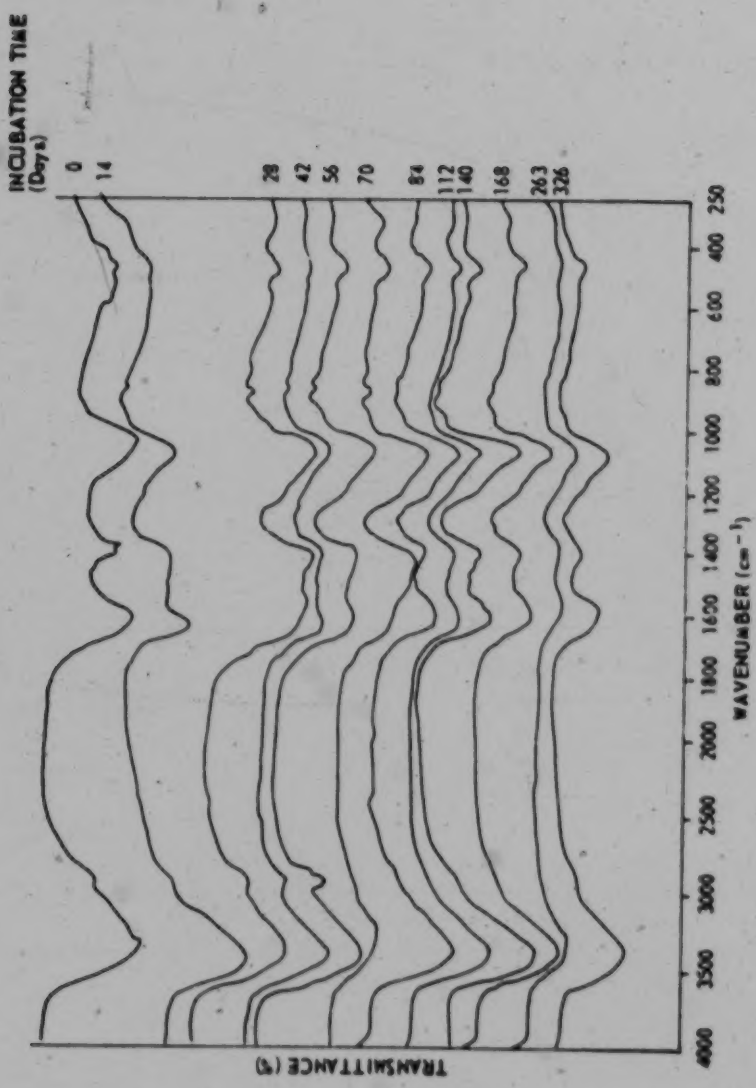


Figure 14. Infrared spectra of the water soluble fraction of raw and decomposing roots of Stipa spartea variety curtiseta incubated at 28°C.

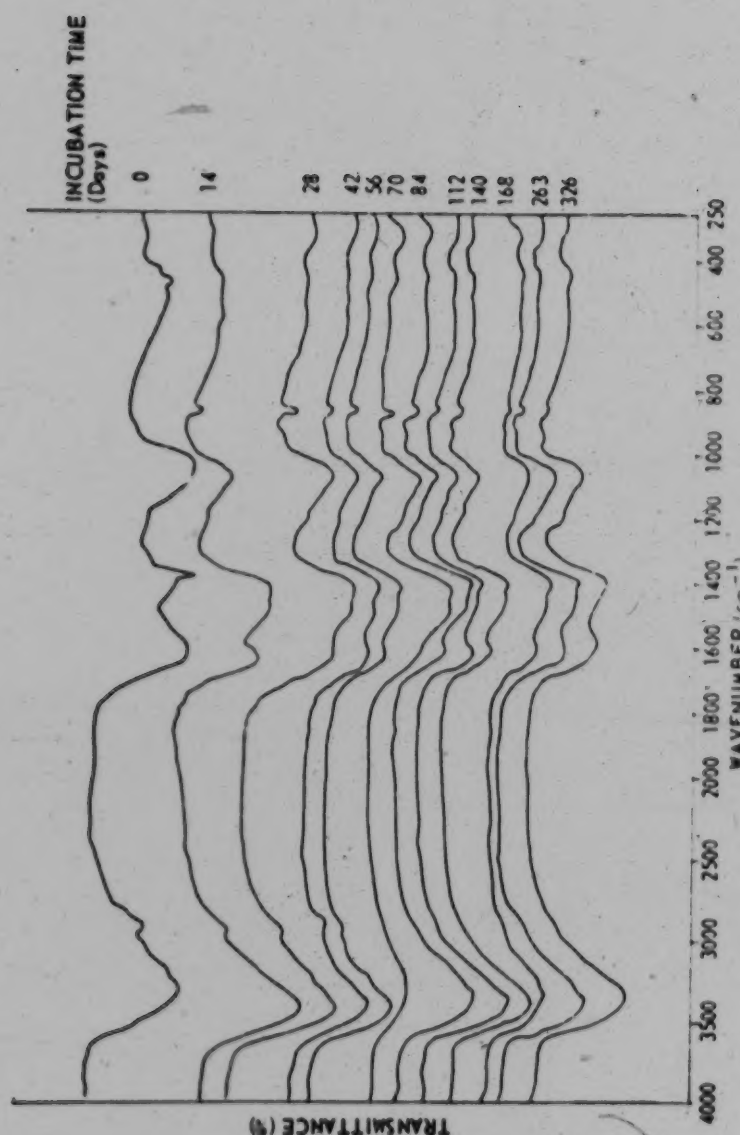


Figure 15. Infrared spectra of the water soluble fraction of raw and decomposing roots of Stipa comata incubated at 28°C.

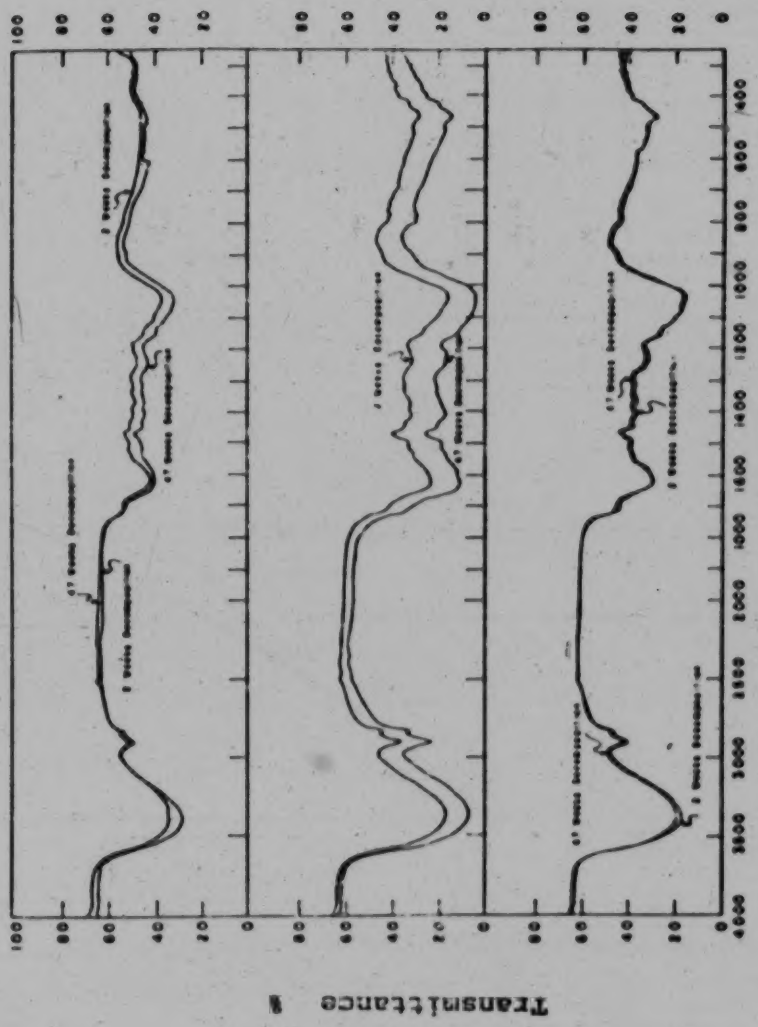


Figure 16. Infrared spectra of the roots of Festuca scabrella (top), Stipa spartea variety curtiseta (bottom) and Stipa comata after decomposition for 2 and 47 weeks at 28°C.

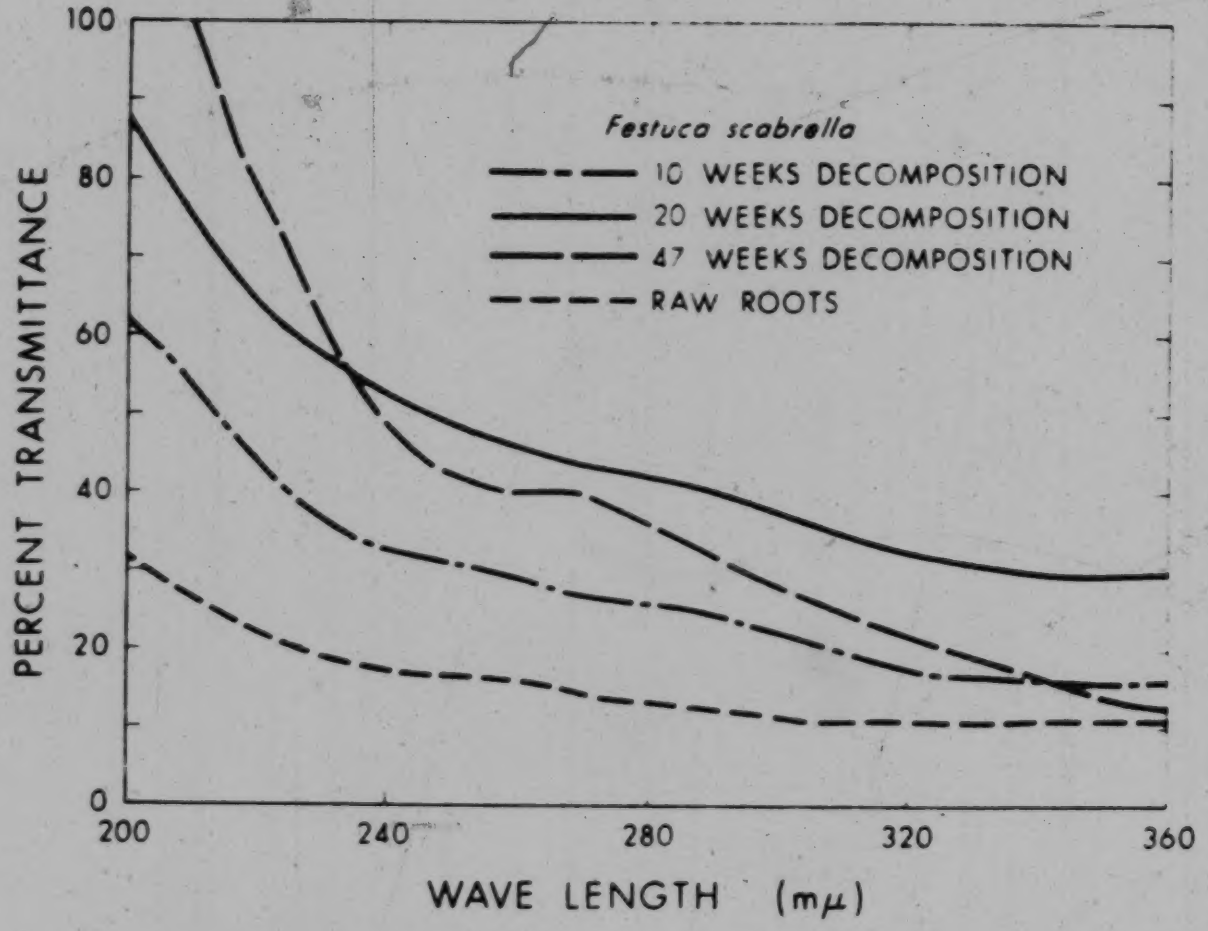


Figure 17. Ultraviolet spectra of the water soluble fraction of Festuca scabrella raw and decomposing roots incubated at 28°C.

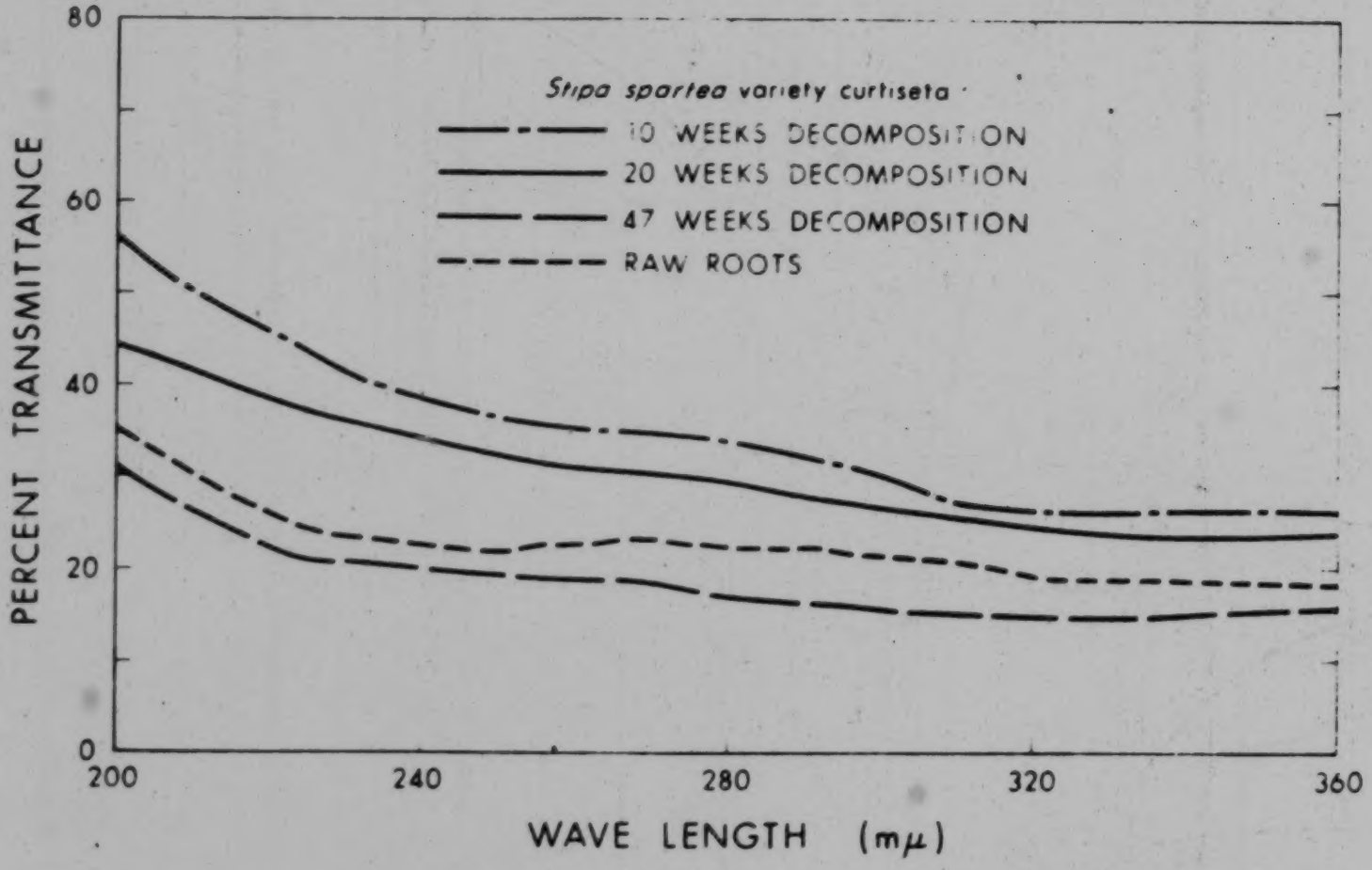


Figure 18. Ultraviolet spectra of the water soluble fraction of Stipa spartea variety curtiseta raw and decomposing roots incubated at 28°C.

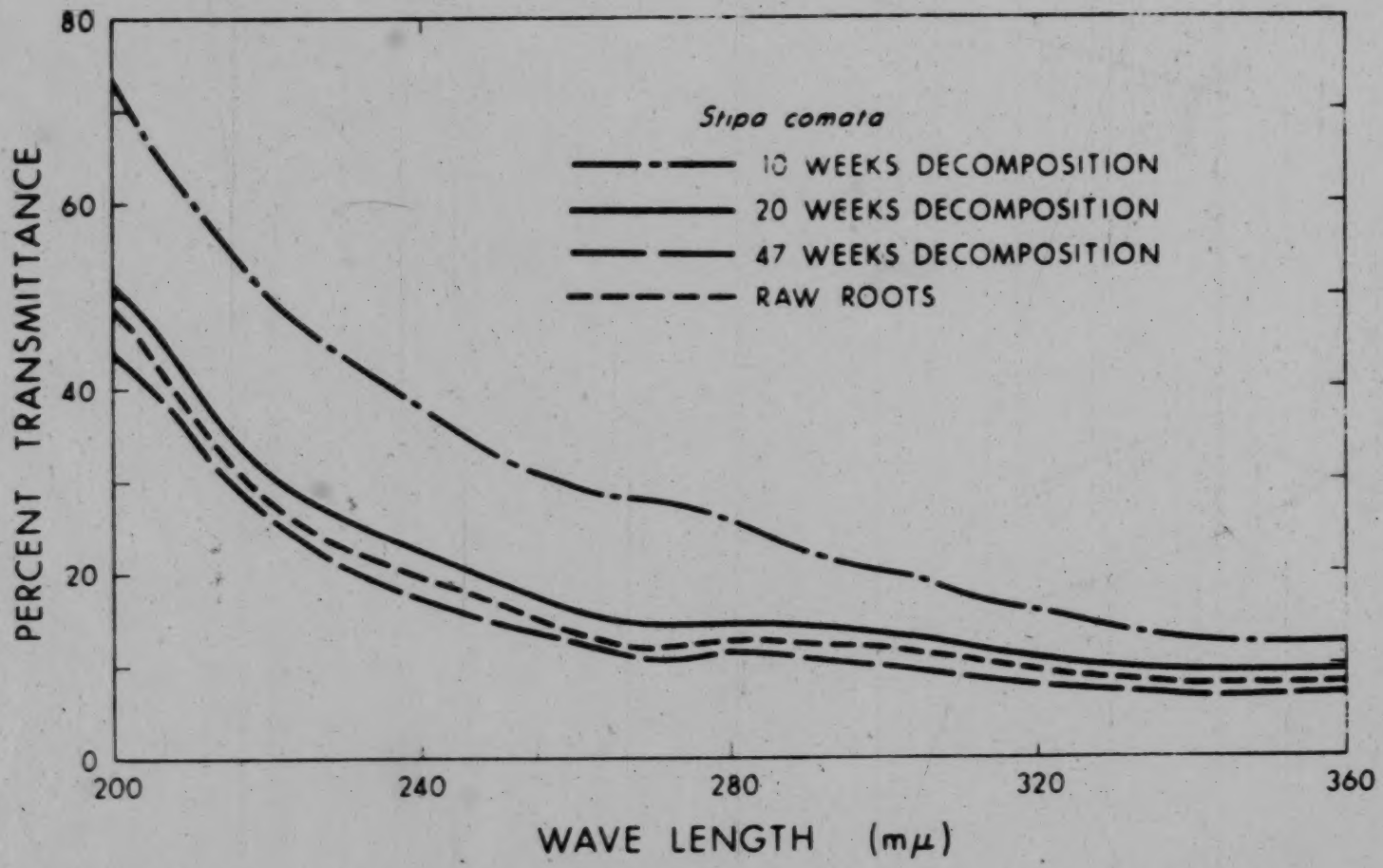


Figure 19. Ultraviolet spectra of the water soluble fraction of Stipa comata raw and decomposing roots incubated at 28°C.

studies of soil humic materials, Felbeck (1965) concluded the ultraviolet spectra to be featureless. There appears to be much more work necessary for proper evaluation of ultraviolet spectra.

Campbell (1967) and Kononova (1966) have shown an inverse relationship between E4/E6 ratios and the degree of increasing complexity or condensation of the aromatic nuclei of humic substances. The simple water soluble materials produced during the decomposition of each species were scanned with visible light to observe possibly the direct relationship between E4/E6 ratios and increasing degree of simplicity in the chemistry of the water soluble fraction (Table 19).

Table 19. E4/E6 Ratios of the Water Soluble Fraction of Raw and Decomposing Roots of Three Grass Species Decomposing at 28°C

Species	Decomposition Time (weeks)			
	Raw Roots	10	20	47
F. scabrella	1.15	1.23	1.29	1.58
S. spartea	1.13	1.04	1.14	1.67
S. comata	1.23	2.33	2.33	2.33

A direct relationship was observed; the E4/E6 ratios of the water soluble fraction of each species increased during

incubation. The E4/E6 ratios of F. scabrella and S. spartea water soluble materials increased continuously during the 47 week incubation period. This trend may indicate continuous chemical changes in the water soluble materials of these two species during incubation. The E4/E6 ratio of the water soluble materials of S. comata attained a maximum after 10 weeks of incubation and remained unchanged for the remaining 37 weeks. This may indicate a situation of chemical alteration in the water soluble fraction of S. comata occurring only in the initial 10 weeks of decomposition. The materials constituting the water soluble fraction of each species may be altered by changes in the residues and microbial suite. Water soluble materials may be utilized by microorganisms as nutrient sources of nitrogen and phosphorus or they may condensate with humic products of decomposition.

DISCUSSION

The chemical analysis of the undecomposed or raw roots of the three grass species studied exposed a difference in the starting materials in terms of elemental distribution and constituent content.

The amount of root weight (dry ash free) lost during the 47 weeks of incubation indicate the root tissues of Stipa spartea variety curtiseta degraded at the fastest rate; the roots of Stipa comata degraded at the slowest rate. The difference between the losses of percent root weight of the fastest degrading tissues and the slowest degrading was only 9 percent. However, the roots and residues of S. comata lost 33 percent of its original carbon whereas Festuca scabrella and S. spartea lost 32 percent and 28 percent, respectively. The greatest percentage loss of carbon occurred in the tissues containing the highest nitrogen at 3.1 percent and the lowest C/N ratio at 18/1. The least percentage loss of carbon did not occur in the tissues containing the least nitrogen or having the highest C/N ratio. The loss of carbon did not give the same indication of decomposition as total ash free weignt loss possibly due to losses of root hydrogen and nitrogen while carbon sources were degraded and synthesized into water insoluble products. Influence of root ash was not studied.

Numerous authors have reported plant degradation rates in relationship to the chemical composition of the degrading tissues. Paul (1970) reports plant and animal tissues adequate in nutrient content and low in lignin degrading rapidly. Bartlett and Norman (1958) report similar results; lignin tending to accumulate in decomposing tissues as the least available major constituent. Kononova (1966, p. 117) reports research by Trusov showing more rapid degradation of highly lignified tissues in the presence of protein substances. Flaig (1964) and Kononova (1966, p. 42) report faster degradation rates in tissues high in structural and soluble carbohydrates. Research by Flaig (1964) and Jenkinson (1966) report the elemental nitrogen content to be of critical importance in degrading tissues. Jenkinson (1966) found the amount of carbon lost from degrading plant residues to be directly related to the residue nitrogen content. Flaig (1964) found nitrogen levels in degrading plant residues limiting microbial activity. To lower the C/N ratio in degrading lignin, Flaig added NH_4NO_3 to accelerate decomposition. Bartlett and Norman (1958), Flaig (1964) and Aleksandrova (1972) have utilized decreasing numbers of methoxyl groups in residues as an indicator of rate of decomposition. Kononova (1966, p. 126) sites increased numbers of acid groups during decomposition as an indication of humification.

Analysis of the original content of lignin and carbohydrates in the three grass species did not predict the observed rates of decomposition in terms of loss of original root weight. The raw roots of S. spartea degraded at the fastest rate despite having the highest lignin content at 38 percent. The roots of S. comata degraded at the slowest rate despite having the highest nitrogen content at 3.1 percent. The methoxyl group content of the roots and residues of all three species oscillated rather than continuously decreased. Many of the results attained in this study disagree with results reported in the literature. The explanation for the observed rates of loss of percent root weight may be found in the changing distribution and total amount of amino acids in the residues of each grass species. Qualitative changes in the amino acid distribution in the residues were expressed as an absolute range or sum of the absolute range of the percentage distribution of each amino acid over a 33 week period. Protein synthesis is genetically controlled (Mahler and Cordes, 1968). It is probable that the protein (hence the amino acid) suite will vary from one type of organism to another. Therefore, changes in the relative distribution of amino acids in decomposing root residues may be considered a reflection of the change in the character of the residual material from one dominated by unaltered root residues to one dominated by microorganisms. The greater the change in relative distribution of amino acids, the greater the amount of alteration one may expect

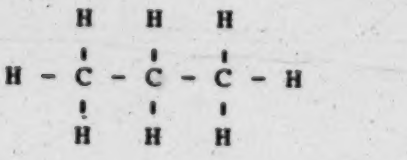
to have occurred in the residues. The greatest change in amino acid distribution occurred with S. spartea, 21.1 percent, which would indicate the greatest change from plant to microbial protein. S. spartea also lost the greatest amount of root weight. Part of this apparent conversion from plant to microbial protein results from the increase in total amino acid content in S. spartea and also in F. scabrella.

The change from a system dominated by the components of plant roots to one dominated by microbial products is consistent with many of the present concepts of humification (Haworth, 1971). The concept that lignin degradation products are converted into humic materials with the incorporation of protein nitrogen is consistent with the observed loss of lignin from S. spartea and build up of amino acid nitrogen. This trend was less pronounced in S. comata (smaller loss of lignin, actual loss in total amino acids and smaller absolute range).

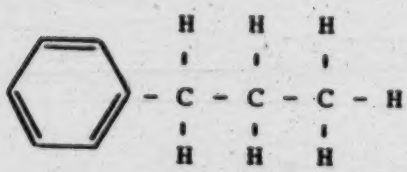
An abundant supply of nitrogen in decomposing plant tissues is considered to favor rapid decomposition and a high level of microbial activity (Jenkinson, 1966). This may be expected to favor those organisms which grow rapidly and hence those functions which can proceed rapidly at the expense of slower functions. Hence, increasing nitrogen supply should accentuate the difference in breakdown rates of easily degraded as opposed to highly

recalcitrant materials. This was observed in this study. S. comata, with the highest nitrogen content and lowest C/N ratio (18/1) lost twice as much of the original carbohydrate component as was the case with the residues of S. spartea and F. scabrella. Also, only 10 percent of the lignin in S. comata roots was degraded whereas 20 percent was lost from S. spartea and F. scabrella.

Kononova (1966, p. 112, 113 and 181) describes the products of plant tissue humification as being highly condensed aromatic molecules of high molecular weight and low aliphatic group content. Wright and Schnitzer (1961) concluded half soil organic material to be present as aromatic compounds. The C/H ratios (weight basis) of four example molecules imply increasing degree of aromaticity and condensation coinciding with an increasing C/H ratio.

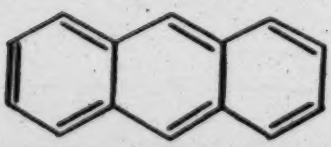


C/H = 36/8 = 4.5



C/H = 108/12 = 9

C/H = 72/6 = 12



C/H = 168/10 = 16.8

The decomposition of the roots and residues of S. comata resulted in the greatest C/H ratio in the residues (10.6/1). The increase may be viewed as an increase in the condensed nature of the organic tissues or merely a loss of aliphatic molecules initially having a low C/H ratio. The latter is likely the case as S. comata lost 61 percent of its initial structural carbohydrate content and only 10 percent of its lignin content. Increasing C/H ratio would appear to reflect the enrichment in lignin and its influence on the system in which S. comata residues were degrading as opposed to S. spartea and F. scabrella in which twice as much lignin was lost. Changes in the C/H ratio of the decomposing residues does not appear indicative of increasing condensation. Loss of aliphatic molecules and groups bonding aromatic units results in a more condensed product of increased aromatic nature. The maximum C/H ratio attained by any of the residues of all three species was 10.6/1 indicating the grass species did not become extensively altered to a more condensed structure but did become more aromatic in nature due to the decomposition and loss of aliphatic molecules.

The E4/E6 ratio of soil humic materials has been inversely related to the degree of condensation of the aromatic nuclei of the decomposing residues; the increasing chemical complexity of the products of decomposition resulting in lower E4/E6 ratios (Kononova, 1966). The E4/E6 ratio of the

water soluble decomposition products of each species was calculated. The maximum E4/E6 ratio was attained by the water soluble fraction of S. comata. The ratio was attained after 10 weeks of incubation and did not change in the remaining 37 weeks. This situation may be indicative of a great amount of degradative activity in the initial 10 weeks of incubation followed by greatly depressed activity for the remaining 37 weeks. During the initial 10 weeks of incubation, 37 percent of the original S. comata structural carbohydrates were degraded while no lignin was degraded. In the remaining 37 weeks, 10 percent of the original S. comata lignin was degraded and 24 percent of the structural carbohydrates. The high E4/E6 ratio in the water soluble products formed from S. comata decomposition is consistent with the small amount of lignin degradation in this species since products of lignin degradation are probably the main contributors to a low E4/E6 ratio in the water insoluble component. The gradually increasing E4/E6 ratio, over the entire 47 week incubation period, of the water soluble materials from the decomposition of the roots and residues of F. scabrella and S. spartea imply a continuous reduction in the amount of condensed water soluble material. This implies either its complete decomposition or its conversion to humified material which would then be located in the insoluble residue. This is consistent with conclusions drawn on the basis of amino acid distribution and loss of lignin.

The incubation period was nearly one year in length with root weight loss varying from 31 to 40 percent and 28 to 33 percent loss of original root carbon. Assuming a 50 percent efficiency, total carbon attacked may have been as high as 50 to 60 percent depending on the amount of synthesized microbial material that was also attacked. Kononova (1966, p. 158) reports a study in which 47 percent of the original weight of timothy roots was lost in 180 days of incubation. This may reflect a difference in chemical composition of timothy or different incubation conditions such as temperature, aeration and nutrient status. The distilled water medium with bubbling atmosphere may not have been conducive to maximum oxidative decomposition. Moistened roots degrading in a humid atmosphere may have resulted in greater oxidative decomposition.

The three soils sampled for the root tissues utilized in this study differed in organic matter content, root density and depth and color of Ah horizon. Differences in the organic matter found at each sample site may be attributed to those factors not held constant in the laboratory study. The factors include climate and its influence on oxidizing and reducing conditions at each site, root density and annual additions of fresh roots to the soil and the influence of the soil mineral fraction on decomposition. The artificial decomposition conditions created in the laboratory did not result in obvious product differences in the three decomposing

grass species. The influence of climate appears to be of extreme importance to the differences in the organic matter status in the soils sampled.

The author wishes to stress a level of caution to be exercised in extrapolation of the results and discussion of the manuscript. The study was conducted using only the roots of three prairie grasses. The results achieved may not be applicable to all plant species and plant members (stems, leaves, roots, etc.) but may serve as guidelines for future studies.

SUMMARY AND CONCLUSIONS

The amount of degradation of the three species studied was similar. A slightly faster rate of decomposition was observed in the roots and residues of Stipa spartea variety curtiseta while the roots and residues of Stipa comata decomposed at the slowest rate.

Conclusions drawn from this study are:

1. The total nitrogen content of the original root tissues could not be used to predict the order of rate decomposition of the three grass species. The C/N ratios of the original tissues did not predict the rate of decomposition of the roots of the three grass species.
2. The grass species with the highest root lignin content did not degrade the slowest; the grass species with the highest root structural carbohydrate content did not degrade the fastest.
3. The increase in root total amino acid content over the decomposition period and the absolute range of the analyzed amino acids were the parameters predicting the order of rate of decomposition in the three grass species studied.
4. Mineralized forms of carbon, nitrogen and hydrogen were lost by the roots and residues of all three species. Losses were probably as CO_2, NH_3 and H_2O.

5. The potential influence of the chemical composition of the undecomposed root tissues on the products of decomposition could not be properly evaluated. The conditions for decomposition and the short length of decomposition time may have masked much of the potential influence of the chemistry of the starting materials. Improved oxidation conditions in the design of the incubation system would have more closely simulated decomposition conditions in the soil and possibly would have enhanced the influence of starting materials.

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APPENDICES

Table 1. Total Weight of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Weight of Roots and Residues (g)		
	F. scabrella	S. spartea	S. comata
Raw roots	18.32	16.97	15.01
2	16.30	14.05	14.45
4	16.06	13.51	13.89
6	16.15	13.31	13.03
8	16.15	13.37	12.04
10	14.25	12.84	11.63
12	12.75	12.77	12.13
16	12.64	10.93	11.30
20	12.62	11.09	11.65
24	12.46	11.10	11.10
37	12.23	11.02	10.68
47	11.96	10.24	10.31

* Total weight on a dry ash-free basis.

Table 2. Ash Content of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Percent Ash Content (%)		
	F. scabrella	S. spartea	S. comata
Raw roots	8.4	15.1	25.0
2	7.6	21.4	17.9
4	7.8	23.1	20.6
6	7.9	18.8	22.3
8	8.2	22.8	21.0
10	7.0	21.4	23.5
12	8.1	19.5	23.6
16	8.1	17.3	24.7
20	7.7	18.9	22.1
24	7.6	18.1	23.2
37	9.0	20.3	24.8
47	10.0	24.0	26.4

Table 3. Lignin Content of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Weight of Lignin (g)		
	F. scabrella	S. spartea	S. comata
Raw roots	5.31	6.49	5.61
2	5.01	7.51	5.91
4	5.21	6.46	5.92
6	5.18	6.40	5.77
8	4.44	5.86	5.49
10	4.03	5.58	5.29
12	3.85	5.57	6.04
16	3.78	4.57	4.68
20	3.86	5.08	5.46
24	3.79	4.81	5.71
37	4.30	4.84	4.98
47	4.18	5.11	5.06

Table 4. Nitrogen Content of Raw and Decomposing Roots of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Weight of Nitrogen (g)		
	F. scabrella	S. spartea	S. comata
Raw roots	.25	.36	.45
2	.20	.34	.42
4	.20	.33	.40
6	.20	.29	.39
8	.21	.31	.37
10	.17	.31	.37
12	.17	.29	.41
16	.16	.25	.41
20	.16	.26	.37
24	.16	.26	.40
37	.16	.29	.38
47	.16	.29	.38

Table 5. Total and Carboxyl Acid Hydrogen of the Water Soluble Degradation Products of Three Grass Species Incubated at 28°C

Species	Type of Hydrogen	Total and Carboxyl Acid Hydrogen (meg. H^+/gm) Decomposition Time (weeks) Raw Roots	10	20	47
F. scabrella	Total	50.6	91.5	147.0	163.4
	Carboxyl	1.2	2.2	2.3	3.5
S. spartea	Total	107.8	109.1	180.8	167.2
	Carboxyl	3.7	2.7	3.4	3.7
S. comata	Total	62.1	129.2	125.0	350.7
	Carboxyl	2.3	3.6	3.1	3.6

Table 6. Decomposition Liquid Medium pH of Three Grass Species Incubated at 28°C

Decomposition Time (weeks)	Decomposition Medium pH		
	F. scabrella	*S. spartea*	*S. comata*
2	8.1	8.0	8.1
4	8.0	7.8	8.0
6	7.8	7.6	8.0
8	7.9	7.9	8.1
10	8.2	7.5	8.1
12	7.9	7.7	8.1
16	8.1	7.7	8.1
20	8.2	7.9	8.3
24	7.9	7.6	7.9